DISSERTATION ON

CLINICOPATHOLOGICAL CORRELATION OF ESTROGEN RECEPTOR ALPHA GENE POLYMORPHISM AND ESTROGEN RECEPTOR STATUS IN BREAST CANCER

A STUDY OF 153 CASES

Dissertation submitted to

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CERTIFICATE

This is to certify that this dissertation titled "**CLINICOPATHOLOGICAL CORRELATION OF ESTROGEN RECEPTOR ALPHA GENE POLYMORPHISM AND ESTROGEN RECEPTOR STATUS IN BREAST CANCER-A STUDY OF 153 CASES"**is the original and bonafide work done by **Dr.N.Hemavathy** under the guidance of Dr.Nalli.R.Sumitra Devi ,M.D.,Professor, Department of Pathology at the Government Stanley medical College & Hospital,Chennai-600 001,during the tenure of her course in M.D.Pathology from May 2010 to April 2013 held under the regulation of the Tamilnadu Dr.M.G.R. Medical University ,Guindy ,Chennai-600032

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I solemnly declare that this dissertation titled "**CLINICOPATHOLOGICAL CORRELATION OF ESTROGEN RECEPTOR ALPHA GENE POLYMORPHISM AND ESTROGEN RECEPTOR IN BREAST CANCER- A STUDY OF 153 CASES** " is the original and bonafide work done by me under the guidance of Dr.Nalli R.Sumitra Devi,M.D.,Professor,Department of Pathology at the Government Stanley Medical College & Hospital ,Chennai -600 0001 ,during the tenure of my course in M.D.Patholgy from May-2010 to April 2013 held under the regulation of the Tamilnadu Dr.M.G.R. Medical University, Guindy,Chennai-600032

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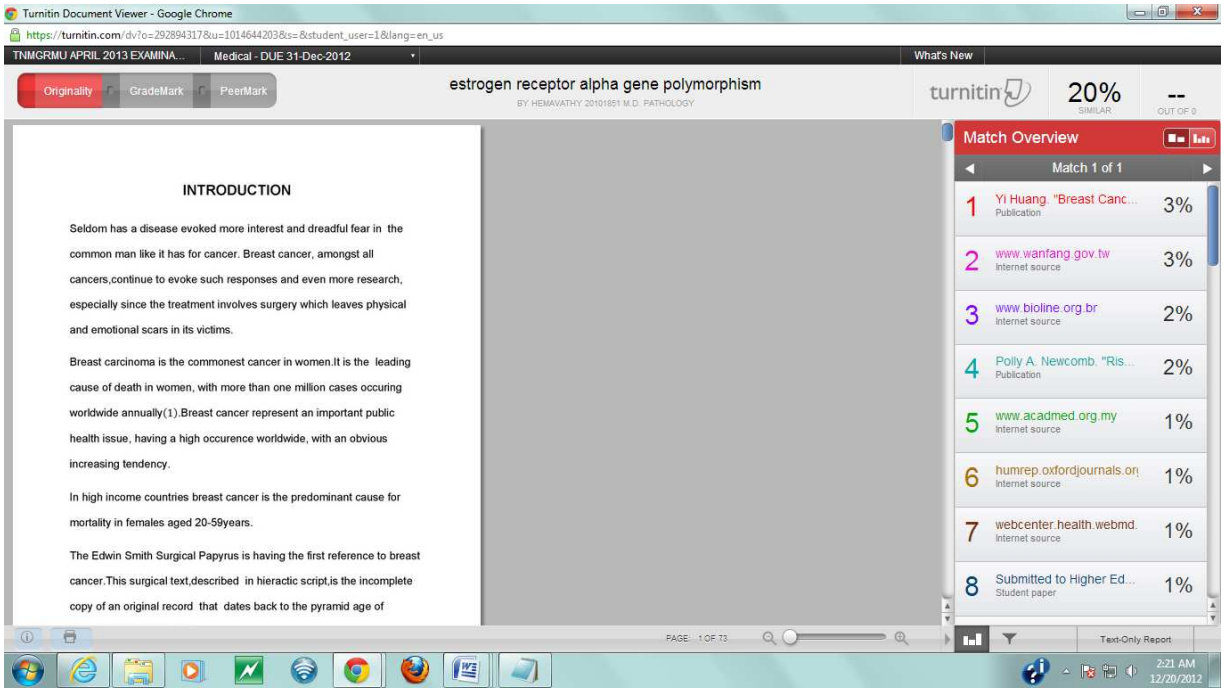
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INTRODUCTION
Seldom has a disease evoked more interest and dreadful fear in the common man like it has for cancer. Breast cancer, amongst all cancers,continue to evoke such responses and even more research, especially since the treatment involves surgery which leaves physical and emotional scars in its victims.
Breast carcinoma is the commonest cancer in women.It is the leading cause of death in women, with more than one million cases occuring worldwide annually(1).Breast cancer represent an important public health issue, having a high occurence worldwide, with an obvious increasing tendency.
In high income countries breast cancer is the predominant cause for mortality in females aged 20-59years.
The Edwin Smith Surgical Papyrus is having the first reference to breast cancer.This surgical text,described in hieractic script,is the incomplete copy of an original record that dates back to the pyramid age of
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INTRODUCTION

ABBREVATIONS

AP-1-Activator Protein -1

DNA-Deoxyribonucleic Acid

EGF-Epidermal Growth Factor

ERE-Estrogen Response Element

ER-Estrogen Receptor

ER-α/ESR1-Estrogen Receptor Alpha

ER-β/ESR2-Estrogen Receptor Beta

GnRH-Gonadotrophin Releasing hormone

Her2/neu-Human Epidermal Growth Factor Receptor 2

hsp-Heat Shock protein

mTOR-Mammalian Target Of Rapamycin

NST-NoSpecial Type

PCR-Polymerase Chain Reaction

PR-Progestrone Receptor

RLN-Regional Lymphnode

RNA-Ribonucleic Acid

SHBG-Sex Hormone Binding Globulin

SNP-Single Nucleotide Polymorphism

WHO-World Health Organisation

INTRODUCTION

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In high income countries breast cancer is the predominant cause for mortality in females aged 20-59years.

The Edwin Smith Surgical Papyrus is having the first reference to breast cancer.This surgical text,described in hieractic script, is the incomplete copy of an original record that dates back to the pyramid age of Egypt(3000-2500BC).(3).

The incidence of cancer has been on rise worldwide .Breast cancer incidence accounts for 16% of all breast cancers,as per the WHO cancer control and prevention programme.It is calculated that 51,9000 women died owing to

breast malignancy in 2004.Inspite of the fact, breast cancer is thought to be a disease of the developed world, majority of breast cancer mortality(69%) , is in developing countries (4). Hence .breast cancer has emerged to be one of the leading cancer killer amongst women worldwide.

Over the last few decades there have been better advances in breast cancer. Earliest detection and skillfull treatment has leading to significant decline in breast cancer deaths. It has also made improved outcome for women living with disease. Breast cancer is no longer seen as single disease but rather a multifaceted disease consisting of diverse biological subtypes with distinct natural history. Breast cancer presents as a varied spectrum of clinial ,pathological and molecular features with diverse prognostic and therapeutic implications.

Estrogen is the steroid hormone, responsible for development and maturation of primary and secondary sexual characteristics in females.(5).Estrogen has an important role in pathogenesis and development of breast cancer(6).

Estrogen receptor is an intracellular protein molecule. They are targets for estrogen action. Estrogen receptor normally resides in cells nucleus ,along with DNA molecules. Estrogen receptor alpha gene polymorphism leads to alteration in estrogen receptor function in breast cancer(7). This study is aimed to ascertain

whether Pvull polymorphism in ER alpha gene has a role in the causation of breast cancer.

AIMS AND OBJECTIVES

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PRIMARY AIMS AND OBJECTIVES:

1. To assess ER-α gene polymorphism is the cause of difference in breast cancer susceptibility among any population
2. To correlate ER-α genotype expression with estrogen receptor expression in cancer cells
3. Whether there is any association between the stage of tumour and specific ER-α genotype

SECODARY AIMS AND OBJECTIVES

1. To correlate the ER-α geneotype frequency and Body mass index among breast cancer patients
2. To analyse ER-α genotype frequency distribution of Pvull polymorphism between pre and postmenopausal women with breast cancer

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Breast anatomy and development provide a foundation for understanding the types of breast cancer that occur and the hormonal factors that influence breast cancer cell growth.

Normal breast tissue:

Human breasts are composed of parenchymal tissues consisting of a branching ductal system radiating from the nipple. The breast parenchyma consists of 15-20 mammary lobes,.Each lobule drains to nipple by lactiferous duct. They are seperated from one another by interlobar connective tissue.Just before entering the nipple,each of the 15-20 main ducts expands into a dilated segment called the lactiferous sinus.Each lobe consists of 30-80 lobules,which contain the milk-producing elements of the breast.The lobules in turn are composed of 20-40 terminal units or acini, which are surrounded by hormonally responsive intralobar connective tissue.(8).The proportion of fat,fibrous and parenchymal tissue vary greatly between individuals and with menopausal status ,weight,number of live births and genetic factors.(9).

Rudimentary breast development begins in utero and then the anatomy undergoes distinct changes at the puberty,during menstruation,with pregnancy and lactation and finally at menopause. At birth ,a female infant has nipples and rudimentary ductal system .At puberty,under the influence of GnRH, anterior pituitary secretes Follicle stimulating hormone(FSH) and Lutenizing hormone (LH) .FSH and LH inturn stimulates ovaries to produce estradiol .

The Estrogens ,primarily 17-estradiol,stimulate the growth and development of breasts. It takes one to two years after menarche before the ovarian follicles are fully mature and begin to ovulate and produce progestrone. Estrogen and progesterone together contribute to the full development of ducts,lobules and alveoli. Fluctuations in estrogen and progesterone levels during a normal menstrual cycle influences breast morphology. During the first half of the menstrual cycle (follicular phase) ,under the influence of FSH and LH,estrogen levels increase and peak halfway through the cycle.Ovulation occurs and then a second peak of estrogen occurs in the second half of the cycle(luteal phase) when the progesterone levels peak. Estrogen and progesterone promote the development of ducts and alveoli respectively in the mammary glands(11).

CARCINOMA OF THE BREAST

Breast cancer develops due to uncontrolled growth of the epithelial cells at the junction of the terminal duct-lobular unit. It has been calculated that most breast cancers need about 5-10 years to develop from a single malignant cell to a tumour of 5-10mm diameter(12).

EPIDEMIOLOGY

Globally,Breast cancer is the most common neoplasm affecting females. It comprises about 25% of all new cases of cancer (13).Breast cancer incidence are low in less developed countries and in Japan than in industralised countries.In 2000,global incidence of breast cancer is over 10 million in which 5.5 million cases are seen in developing countries.(14).

Cervical cancer incidence are higher in developing countries than breast cancer,the reverse holds true in developed countries..

In India ,breast cancer is the second most common cancer in women after cervical cancer. However in Indian metropolitan areas, breast cancer became most common cancer than cervical cancer (15)

Breast cancer incidence rate shows geographical variation largely because of socio-economic,reproductive,hormonal,nutritional and genetic factors. Highest incidence rates are seen in NorthAmerica and Europe.Asia and Africa has the lowest incidence rates of breast cancer(16).

After remaining constant for many years,the incidence of breast cancer began to increase .This is due to detection of increased number of cases by means of introduction of mammographic screening in early 1980's.(17).

The main aim of screening is the detection of small predominantly ER positive invasive carcinomas and insitu carcinomas .DCIS is almost exclusively detected by mammography ,providing an explanation for sharp increase in the diagnosis of DCIS since 1980.

From 2001 to 2004 ,the incidence of Estrogen receptor positive invasive breast cancer has raised. The reason for this trend is multifactorial. This may be attributed that ,in 2002 many women stopped using hormone replacement therapy .

During the 1980's the number of women dying of breast cancer remained constant ,while the incidence of breast cancer was increasing. Since 1994,the breast cancer mortality rate for all women has slowly declined from 30% to 20%.The decrease is attributed to the detection of clinically significant cancers at a curable stage due to screening,as well as better and effective treatement modalities(18)

ETIOLOGY AND RISK FACTORS

Breast cancer is a multifactorial disease ,meaning a variety of factors contribute to the biologic processes involved in carcinogenesis. Some of these

factors are genetic changes in oncogenesis and in tumour suppressor genes, growth factor imbalances,enzyme production and telomerase activity. These genetic changes interact with non genetic factors such as environment, nutrition and other lifestyle risk factors leading to cancer. By identification of modifiable risk factors and controlling them, the risk of breast cancer has been lowered.

Any factor, such as ovarian hormones and growth factors, that increases cellular proliferation in breast epithelium raises the risk . Increased cell proliferation increases the opportunities for spontaneous genetic damage leading to breast cancer risk.(19).

Reproductive Risk Factors:

Early Menarche and late Menopause:

Early age at menarche increases risk of breast cancer .In general for every one year delay breast cancer frequency decreases by 10-20%.

Both age of onset of menarche and regular cycles influence the risk of breast cancer. Breast cancer risk may be explained by effect of early menarche on estrogen level.(20)

Women with surgically induced menopause have been shown to have reduced risks of breast cancers compared to women whose menopause occurred

naturally.In comparison with women whose menopause occurs between the ages of 45 and 54(relative risk 1),women with late menopause at age more than 55 years have a relative risk of 1.48.Increased risk of breast cancer in late menopause is due to long menstrual history and ovarian function (21).

Parity

Early age at first pregnancy decreases risk of breast cancer.17-41% reduction in breast cancer risk is seen in parous women when compared to nullipara(22).These reduction is not immediately found in parous women. Actually the risk is increased in the first ten or more years following pregnancy.It may be explained by proliferatve changes in pregnancy.Breast cancer risk is decreased by 7% for every childbirth thereafter. This reduction is attributed to maximum differentiation in breast parenchyma in which further DNA damage does not takes place.

Age at First Live Birth

Early first pregnancy leads to maturation of terminal ductal lobular unit of breast thereby reducing risk of breast cancer. Hence.women who are more than 35 years of age had 60% increase in breast cacer than those are less than 18years of age at first pregnancy (23).

Breast feeding

Breast feeding further reduces risk of breast cancer in parous women. There is about 12% decrease in relative risk of breat cancer development for women who breast feed for one year. (24).This reduction percentage is increased upto 50% in high parity females.

Breastfeeding is thought to decrease breast cancer risk by lessening the total number of menstrual cycles and consequently cumulative ovarian hormone exposure .

Hormones

Reproductive risk factors are well known to influence breast cancer risk by modulating endogenous hormone levels. In the following sections, the relation between serum hormonal levels and breast cancer risk will be discussed.

Estrogens and Androgens

Estrogen increases cell proliferation in the breast. In premenopausal women, nearly all estrogen is of ovarian origin. After menopause, direct ovarian production stops and most estrogen is derived from the aromatization of adrenal androgens .

Estradiol and estrone sulfate are the types of estrogen implicated in breast cancer development.The 17β estradiol is the most functionally active form of

estrogen from puberty till menopause. Estradiol circulates in the blood either as free hormone or bound to sex hormone –binding globulin(SHBG) and albumin. Free estradiol or estrogen which binds to albumin are functionally more active forms.Major circulating estrogen is estrone sulfate. They are the majour resource of estrogen from adipose tissue in postmenopausal females.

Androgens such as testosterone and androstenedione can be aromatized into estrogens, either in the ovaries or in adipose tissues.Estrogen is derived directly from ovarian and adrenal synthesis as well as from the peripheral conversion of androstenedione.

Breast cancer risk is directly proportional to the levels of serum concentrations of sex hormones including total and free estradiol, androstenedione, and testosterone (25).

Serum estradiol levels have been shown to be be less in Asian women regardless of menopausal status. These differences seen in low risk population may be due to reduced number of ovulatory cycles as a result of late age at menarche, higher parity,frequent breastfeeding ,breast feeding for longer durations, and early age at menopause.

In postmenopausal women , weight is directly propotional to plasma levels of estrone and estradiol,as well as unbound estradiol to SHBG. Hence

postmenopausal obese women have greater risk of breast cancer development than in nonobese women.

Hormone Replacement Therapy

Invasive breast cancers occur more among the current users of hormone replacement therapy especially those who have used more than five or more years. However the risk of breast cancers is no higher among former users who have stopped taking hormones more than 5 years previously, than the risk among never users. Hence the major consequence of hormone replacement therapy is promotion of cancer growth rather than direct genotoxic effect.

Breast cancer risk is increased by 2.3% for each year among women using hormone replacement therapy currently ,or who have stopped within one to four years. The relative risk for breast cancer is 1.35 for women who had used hormone replacement therapy for more than 5 years.(26)

Anthropometric Risk Factors

The correlation between weight and breast cancer risk differs according to menopausal status. Increased weight or BMI has been shown to lessen breast cancer development in premenopausal but increases risk in postmenopausal women.

Several hypothetized mechanisms exist to explain the low risk of breast cancer in obese premenopausal women . Obese premenopausal women have decreased progesterone levels because obesity may cause anovulation and a reduced progesterone secretion in the luteal phase . Also, leptin levels, which increase with increasing fat stores, inhibit ovarian estrogen production, and may thereby derease breast cancer development in obese premenopausal women .

Obesity increases breast cancer risk in postmenopausal women by increasing levels of endogenous estrogen (27). The principal source of estrogen in postmenopausal female is the conversion of androstenodione to estrone in adipose tissue. Also, sex-hormone-binding globulin levels fall when BMI is increased, thus increasing the levels of free estradiol . In addition, obesity may increase the concentration of several circulating cytokines, which stimulate the activities of the enzymes, involved in the synthesis of estrogen .

Family History of Breast Cancer / Genetic Factors

Family history of breast cancer is one of the most well-established risk factor for breast cancer.Some family history are important,while others are of little consequence.Most women who have relatives, who have developed breast cancer postmenopausally are not genetically predisposed to breast cancer and their increased risk is low.On the other hand, a women who has first degree relatives

with breast cancer have substantially increased risk of breast cancer. Risk is about 1.5-2 times above in the woman who have no affected first degree relative.The risk may be further increased to 6 if more than one, first degree relative has been affected. Cancers develop in these population at an earlier age in their mother or sister. Also they have inherited DNA mutation of BRCA 1 or BRCA 2 gene, that increases the risk of breast cancers.

Lynch, distinguishes familial breast cancer from hereditary breast cancer. Familial breast cancer is defined as “Family having more than two first degree relatives with breast cancer in the absence of hereditary breast cancer”.Hereditary breast cancer is defined as”Pattern within a particular family having Mendelian segregation of breast cancer”. The former are probably events that may happen,by the laws of probability to cluster in a family,while the latter cancers are likely the results of inheritance of abnormal DNA.(28)

Genetic factors have a role in approximately 5% of all breast cancer cases,. But the risk percentage increases to 25% of cases below 30 years of age. Several genes are implicated in l breast cancer development. BRCA 1 gene located on chromosome 17 and BRCA 2 present on chromosome 13 are associated with majority of inherited breast cancers. 2-5% of breast cancers are hereditary .BRCA 1 and BRCA 2 are the tumour suppressor genes with numerous important

cell functions.It includes gene transcription, regulation of cell cycle check points and DNA repair.

OTHER GENES:

Many genes other than BRCA are involved in breast cancer risk. Women with Li-Fraumeni syndrome have increased risk in development of early onset of many cancers including breast cancer.This syndrome is due to mutations in p53 tumour suppressor gene In Ataxia telengiectasia,there is 100 fold increase in breast cancer risk in women.It is the autosomal recessive syndrome due to DNA repair defect.Women with Cowden disease having mutation in the PTEN tumour suppressor gene develop breast cancer by 50 years of age.

Hence,Breast cancer may also develop due to alleles with low to moderate penetrance.They confer lesser risk,but attributable risk is more when it is common in the population.Breast Cancer Association study implies that larger samlple size is required to clarify association of polymorphism with breast cancer .(29)

Genetic susceptibility due to both high and low penetrance gene mutation, together with interaction of environmental factor leads to increased incidence of breast cancer.

Medical History

Benign Breast Diseases

Certain types of benign breast diseases have increased risk of breast cancer. Women with benign breast disease without hyperplasia have a 1.5 fold increased risk of breast cancer compared to normal population.. The risk of breast cancer among women with hyperplasia varies with presence of atypia or not.

Atypical hyperplasia increases, 2.6 fold risk of breast cancer as compared to 1.8 fold increased risk in hyperplasia without atypia.

The breast cancer risk associated with benign breast disease differs by menopausal status. Atypia in premenopausal women have higher relative risk of breast cancer than in post menopausal women.(30)

Mammographic Density

Mammographic density is a strong risk factor for breast cancer. It represents connective and epithelial tissues in the breast ,whereas the dark radiolucent areas on the mammogram are primarily fat. Women with highest mammographic density are 4-6 times more likely to develop breast cancer than very low density.(31)

Ionizing Radiation

Ionizing radiation is has increased risk of breast cancer.Information on radiation and breast cancer risks has come mainly from epidemiological studies of atomic bomb survivors or women exposed to radiation for diagnostic or therapeutic reasons. Relative risks vary from 1.2 -2.4 and are related to both total dose and age at exposure. Younger women had greater risk than older women(32). The effect of very low doses such as those incurred in occupational exposures is uncertain . Because of the low doses involved in screening mammography (200 – 400 mrad) and of the finding that older women were less susceptible to ionizing radiation, the benefit risk ratio for older women would still be large .

Socioeconomic Status

Higher socioeconomic status has a role in breast cancer . Developed countries have much higher breast cancer rates than developing countries. This correlation between socioeconomic status and breast cancer risk has also appeared at both the,individual and community level.The higher breast cancer risk among well-educated women appears to be attributable to greater exposure to breast cancer risk factors such as later age at first pregnancy, having few or no children, and more frequent use of oral contraceptives and hormone therapy

RISK FACTORS FOR BREAST CANCER AND THEIR RELATIVE RISKS(33)

S.No.	RISK FACTOR	COMPARISON CATEGORY	RISK CATEGORY	RELATIVE RISK
1.	Age at menarche	16 years	Younger than 12 years	1.3
2.	Age at menopause	45 to 54 years	After 55 years	1.5
3.	Age at when first child born alive	Before 20 yeats	Nulliparous or older than 30 years	1.9
4.	Benign breast disease	No biopsy or fine needle aspiration	Any benign disease	1.5
			Proliferative disease	2.0
			Atypical hyperplasia	4.0
5.	Family history	No 1^{st} degree relative affected	Mother affected	1.7
			Two first degree relative affected	5.0
6.	Obesity	10 th percentile	90^{th} percentile	1.2

7.	Alcohol use	Non drinker	Moderate drinker	1.7
8.	Estrogen replacement therapy	Never used	Current use >3 years	1.5

Diagnostic modalaties

Mammography

Screening mammography, is used to detect cancer in asymptomatic women. Diagnostic mammography is used to evaluate to

1. Patients with breast symptoms or complaints,such as nipple discharge or a palpable mass
2. Patient who have had abnormal results on screening mammography or
3. Patients who had under went breast conservation therapy.

The diagnostic examination is tailored to the individual patients specific abnormality .

Digital mammography (Also called full-field digital mammography, or FFDM) –

FFDM is anew technology that was recently approved by the FDA for breast cancer screening and diagnosis**.** They capture the images which are processed on a computer and then viewed.

BI-RADS diagnostic categories —

After analysing the mammographic images, radiologists classify findings into a final assessment category .The Breast Image Reporting And Data System(BIRADS) ,final assessment classification was developed by the American College of Radiology to standardize mammographic reporting. Follow up recommendation are made based on the final assessment category.BIRADS 0 or incomplete final assessment require additional imaging to render or resolve, or define an abnormality on screening examinations.

ULTRASONOGRAPHY

Ultrasound can be used to differentiate between solid and cystic breast masses that are palpable or detected mammographically. In addition, ultrasound evaluation of the axilla can be used to detect lymph nodes that are suspicious for axillary metastases. Ultrasound provides guidance for interventional procedures of suspicious areas in the breast or axilla.

BREAST MRI

The sensitivity of MRI for breast carcinoma is between 88 and 100 percent .Invasive breast cancer shows contrast enhancement on MRI. Because MRI is so sensitive, it was assumed that preoperative MRI would estimate the extent of disease, more accurately than conventional imaging, thereby improving surgical planning (eg, prompting a change to mastectomy when breast conserving therapy had been previously considered and enabling surgeons to obtain clean margins in breast conserving surgery).

Fine needle aspiration cytology

FNAC of a palpable breast mass can easily proceed in outpatient setting. They are used to differentiate solid and cystic lesions.

The combination of diagnostic mammography,ultrasound and fine needle aspiration biopsy achieves almost 100% accuracy in diagnosis of breast cancer.

Biopsy

Core Needle biopsy

Core needle biopsy is, tissue sample obtained from the mass by using hollow needle. The advantages of core biopsy are low complication rate, avoidance of scarring and low cost

Open biopsy

An open biopsy is recommendedonly only in patients who have been appropriately investigated by imaging,FNAC,and or by core needle biopsy

Proliferative Biomarkers

High proliferative decreases of breast cancer is associated with favourable response to chemotherapy.The main obstacles to use proliferative markers are

1.Poor standardisation of detection methods

2.Vaguely defined cutoff values

3.Requirement of fresh fozen tissue

Proliferative biomarkers are

1.**Measurement of cells in S phase:**

Unfavourable prognosis of breast cancer patients are seen when S phase fraction is assessed by fresh or frozen material . But several studies done to assess the prognostic value by DNA flow cytometry lacks standardised procedures,sufficient power and predifined cutoff values. There is also high tumour heterogenity of the S phase fraction .Therefore it cannot be recommended

for routine prognositc assessment. Another disadvantage of this method is requires large quantity of tumour material, making it inappropriate for smaller tumours identified through mammographic screening.

2.**H-Thymidine Labeling Index**

H-thymidine labeling index was one of the proliferative used in breast cancer. Cells undergoing DNA replication is measured by H-thymidine uptake using autoradiography. Thymidine labeling index represents ratio between the number of labelled and counted cells .A similar approach uses IHC technique and halogenated analogue -Bromodeoxyuridine.Limitations of this technique are the requirement of fresh frozen tissue,the time requirerd to complete the assay and use of radioactive tracers.

3.**Thymidine Kinase**

Thymidine kinase activity is measured by radioenzymatic assay Thmidine kinase is an enzyme that catalyses the phosphorylation of deoxythmidine to deoxythymidinemonophosphate. Its activity is highest in G1-G translation check point and then reduced in G2 phase of cell cycle. In breast cancer ,the fetal isoform of Thymidine kinase is present in high levels in the cytoplasm and regulates the cell cycle.

4.**Ki67**

Ki 67 is a nuclear antigen which is present in mid G1,S,G2 and the entire M Phase of the cellcycle .Overexpression of Ki67 correlates with proliferative activity metastases and overall survival.

5.MIB1

Similar to Ki67, MIB1 is a nuclear antigen that can be labelled using immumohistochemistry. It can be performed on formalin fixed and paraffin embedded tissue. A good concordance is seen between Ki67 and MIB1 assessment.

6.Cyclin A

Cyclins are proteins that regulate cell cycle. Cyclin A is expressed mainly in the late S,G2 and M phases of the cell cycle. It has been associated with poor prognosis.

7.Cyclin E

Cyclin E regulates G1 phase progression and entry into S phase.There are two different proteins,cyclin E1 and E2.,that are coded by 2 different genes with 47%

homology. Cyclin E1 is determined by immunohistochemistry ,western blot and RT-PCR.Elevated levels of cyclins increases risk of breast cancer related death.

8.Cyclin D1

The family of cyclin D consists of atleast three different cyclins that regulate progression into G1 phase. The function of cyclin D1 is to bind to the cyclin dependent kinases 4 & 6 and phosphorylate downstream proteins .These complexes can sequester cyclin D kinase inhibitors .Cyclin D1 acts as a cofactor for ER alpha in a ligand independent manner. The concentration of Cyclin D1 is the highest during mid G1 phase and then gradually declines. Overexpression of cyclin D1,mRNA,protein and amplification to be 15% in breast cancer.They are found to be associated withre ER positive and well differentiated tumours .The most common method of detection of cyclin D1 expression is immunohistochemistry.

9.p27

p27 is a cyclin dependent kinase inhibitor that acts in the nucleus.It is mobilized by antiproliferative signals,such as cell to cell contact and transforming growth factor beta.It can be assessed by immunohistochemistry.In majority of the studies p27

was positively correlated with ER expression and inverse correlation with the grade.In BRCA1/2 mutated tumours,low level of p27 are seen.

10.Topoisomerase II alpha

Topoisomerase II are DNA binding enzymes with nuclease,helicase and ligase activity. Topoisomerase IIbeta is not cell cycle dependent whereas topoisomerase IIalpha is cell cycle dependent and is highest inG2/M transition.Coamplification of topoisomerase II and Her-2 are associated with increased sensitivity to anthracyclines. It is also used as a prognostic marker for overall survival of breast cancer patients independent of therapy

11.Urokinase plasminogen activator(uPA) and Plasminogen activator inhibitor(PAI-1)

uPA and PAI-1 levels are associated with breast cancer recurrence and survival.They also predict the hormone therapy and specific types of chemotherapy response.

The breast cancer markers that are most important in determining therapy are estrogen and progesterone receptor and Her-2/neu

Staging of breast cancer

TNM staging of tumour is based on size of primary tumour,regional lymph node and distant metastasis.

American Joint Committee on Cancer (AJCC) staging system provides guidelines for breast cancer patient according to the prognostic status.The AJCC has designed staging by TNM classification.(34)

TNM STAGING

Primary tumour(T):

TX:Primary tumour cannot be assessed

T0:No evidence of primary tumour

Tis:Carcinoma insitu;DCIS/LCIS/Pagets

T1: Tumour size(2 cm or less).

T1a:less than 0.1cm microinvasion

T1b:more than 0.5cm but less than 1cm

T1c:more than 1cm but less than 2 cm

T2: Tumour size 2-5cm

T3:Tumour size more than 5cm

T4:Tumour of any size with direct extension to chest wall and or to the skin(ulceration or skin nodules)

T4a:Extension to chest wall,not including only pectoralis muscle invasion/adherence

T4b:Ulceration and/or ipsilateral satellite skin nodules and/or edema)

T4c:both of the above(T4a and T4b)

T4d:Inflammatory carcinoma

Regional lymph nodes(N)

NX: (RLN) cannot be assessed

N0: No regional lymphnode metastasis

pN0(i-): No ‘RLN’ metastasis identified histologically, negative IHC

pN0(i+):Malignant cells in ‘RLN’ less than 0.2 mm (detected by H&E or IHC)

pN0(mol-):No RLN metastasis histologically, negative molecular findings(RT-PCR)

pN0:Positive molecular findings (RT-PCR) but no RLN metastasis detected histologically or by IHC

pN1:Micrometastasis: or metastasis to 1 to 3 axillary lymphnodes and/or internal mammary nodes with metastases detected by sentinel lymph node biopsy but not clinically detected

pN1m1:Micrometastasis(greater than 0.2 mm and /or more than 200 cells but none greater than 2.0mm)

pN1a:Metastases in 1 to 3 axillary lymphnodes,atleast one metastases greater than 2.0 mm

pN1b:Metastases in internal mammary nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not detected clinically

pN1c:Metastases in 1 to 3 lymph nodes and in internal mammary nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not detected clinically

pN2:Metastases in 4-9 axillary lymph nodes; or in clinically detected** internal mammary nodes in the absence of axillary lymph node metastases

pN2a:Metastases in 4-9 axillary lymph nodes(atleast one tumour deposit greater than 2.0 mm).

pN2b:Metastases in clinically detected internal mammary nodes and in the absence of axillary LN metastasis

pN3:Metastases in 10 or more axillary lymph nodes;or in infraclavicular (level3) lymph nodes;or in clincally detected ipsilateral internal mammary in the presence of 1 or more positive level 1,2 axillary lymph nodes;or, in more

pN3:Metastases in 10 or more axillary lymph nodes;or in infraclavicular (level3) lymph nodes;or in clincally detected ipsilateral internal mammary in the presence of 1 or more positive level 1,2 axillary lymph nodes;or, in more than 3 axillary

lymph nodes and in internal mammary lymph nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected or in ipsilateral supraclavicular lymph nodes

pN3a: Metastases in 10 or more axillay lymph nodes(at least one tumour deposit greater than 2.0mm);or metastases to the infraclavicular (level3 acillary lymph nodes and in internal mammary lymph nodes) nodes

pN3b:Metastases in clinically detected ipsilateral internal mammary lymph nodes

Distant metastases(M):

MX: metastasis cannot be assessed

M0:No distant metastasis

M1:Distant metastasis present (includes metastasis to ipsilateral supraclavicular lymph nodes) age groupings

STAGING:

Stage groupings:

Stage 0

Tis,N0,M0

Stage 1

T*1,N0,M0

*T1 include T1mic

Stage 2A

T0,N1,M0

T1*,N1**,M0

T2,N0,M0

*T1 includes Tmic

.**The prognosis of patient with pN1a disease is similar to that of pN0 disease

Stage 2B

T2,N1,M0

T3,N0,M0

Stage 3A

T0,N2,M0

T1*,N2,M0

T2,N2,MO

T3,N1,M0

T3,N2,M0

*T1 includes T1 mic

Stage 3b

T4,Any N,M0

Any T,N3,M0

Stage 4

Any T,Any N,M1

Histological Grading of Breast Cancer

Histologic grading based on

1. Cell mitosis
2. Tubule formation
3. Nuclear pleomorphism

The grading criteria are as follows:

Modified Bloom Richardson histologic scoring criteria(35)

1.**Tubule formation**	Score
>75%	1
10 to 75%	2
<10%	3
2. Nuclear pleomorphism	
Small uniform cells	1
Moderate increase in size and variation	2
Marked variation	3
3.Number of Mitosis (Microscope Nikon 40x objective)	
Upto 5	1
6-10	2
	3

More than 11

Nottingham Modification of Bloom Richardson grading system:

GRADE	DESCRIPTION	SCORE
Grade 1	Well differentiated breast cells. Cells generally appear normal.not growing rapidly. Cells arranged in small tubules.	3,4,5
Grade 2	Moderately differentiated breast cells.Have characteristics between Grade 1 & 3 tumours	6,7
Grade 3	Poorly differentiated breast cells.Cells do not appear normal and tend to grow and spread aggressively.	8,9

FIVE YEAR SURVIVAL RATE:

STAGE	5YEAR SURVIVAL RATE
0	100%
I	100%
IIA	92%
IIIB	81%
IIIA	67%
IIIB	54%
IV	20%

Estrogen receptor

Estrogen receptors are members of the steroid receptor superfamily. Two isoforms of ER such as ER alpha and ER beta have been identified. Although ER alpha and ER beta have only 30% overall sequence similarity,their DNA binding and ligand binding domains are highly homologous ,suggesting that the two receptors likely share similar ligands and DNA binding activity. Most of the knowledge about ER activity and function has been obtained from studies on ER alpha. Much less is known about ER beta(36).

The ER is a nuclear transcription factor that regulates the expression of ,number of genes involved in regulation of transcription and differentiation. Binding of ligand to ER results in a ligand-ER complex that subsequently induces an ER conformational change,dissociation of chapreones such as hsp90 and hsp70 and receptor dimerisation.Activated ER dimers can bind to the Estrogen receptor elements(ERE) of target genes and regulate their transcription. Some nuclear proteins interact with ER and function as coactivators or corexpressors of ER.

Because of the pivotal role that ER plays in breast cancer progression, development of specific agents, targeting ER or its ligands has become an

important strategy for breast cancer treatment. Applied endocrine therapies, include depletion of the ligand ,estrogen ,steroidal antiestrogens that destroy ER and selective ER modulators.

Unfortunately denovo or acquired hormone resistance is a feature of some breast cancers. Approximately 30% of breast cancers lack ER gene expression. Tumour lacking ER protein are usually associated with higher growth rate, poor differentiation and worse clinical outcome. Thus ER expression has been a possible prognostic factor for early breast cancer patients. Genetic changes that account for the loss of ER in breast cancer include deletions, insertions ,point mutations or rearrangement of ER gene.

ER alpha which is located on chromosome 6q25.1, contains 595 aminoacids with central DNA binding domain with carboxy terminal hormone binding domain.ER beta which is located on chromosome 14q23.2 ,lacks large portion of carboxy terminal F domain.

Tissue distribution of ERs:

The distribution of two receptors overlap in breast, endometrium, bone, prostate, epididymis, central and peripheral nervous system(37).

Liver and white adipose tissue show ER alpha expression alone.

Kidney,Bladder,Intestine ,ovary and show only ER beta expression alone.

ER alpha gene polymorphism that are studied frequently are Pvull and Xbal gene polhymorphism .They have been associated with breast cancer,prostate cancer ,neurodegenerative disorder such as Alzheimers disease .

Screening for intron 1 single nucleotide polymorphism in ER alph gene is commonly carried out by means of PCR amplification followed by RFLP analysis. Pvull is used for T397C and XbaI is usedfor C351G.PCR is utilized to amplify TA dinucleotide repeat using labelled -deoxy cytidine triphosphate(38).Amplified products are then seprated and analysed on denaturing polyacrimide sequencing gel.

IMMUNOHISTOCHEMISTRY

IMMUNOHISTOCHEMISTRY

Immunohistochemistry involves two disciplines immunology and histology.Immunohistochemistry is used to determine expression of particular antigen and its microanatomic location in the tissue.

Immunohistochemistry was started in 1940 when Coons developed an immunofluorescence technique to detect corresponding antigen in frozen sections.

Taylor and collegues in 1974 showed it was possible to demonstrate antigens in routinely processed tissue.Antigen retrival technique was introduced by Shi and associates in 1991.Antigen retrival technique is a simple method that involves paraffin processed sections at high temperature before IHC staining.

The use of antibody in IHC depends on the sensitivity and specificity of the antigen –antibody reaction.

Blocking non-specific background staining

Background staining is due to either non specific binding or presence of endogenous enzymes.Non specific binding with polyclonal primary antibody is minimized by preincubating sections with serum from same species on optimal working dilution.

Endogenous enzymes such as peroxidase seen in normal and neoplastic tissues is abolished by peroxidase blocking or by using alternate systems such as immunogold technique.

Methods suggested to overcome endogenous activity include incubation in methanol containing 0.5% hydrogen peroxide for 10 minutes at room temperature(almost complete abolition of endogenous peroxidase activity).Endogenous alkaline phosphatase is blocked by addition of 0.1M concentration of levamisole to the enzyme substrate solution.

Detection systems:

Antibodies are labeled or flagged by some method to permit visualization .These includes fluorescent substances, enzymes forming colored reaction with suitable substrate(light microscopy) or heavy metals(electron microscopy)

Methods of IHC:

Direct labeling method:

Antibody is attached with a label by chemical means and directly applied to tissue sections. It is a rapid and easy procedure and carries the disadvantage of usinf multiple antigens which require seprate incubation with respective antibodies.

Indirect labeling method:

Enzymes are labeled with secondary antibody ,which is produced against primary antibody. This method is more sensitive and easy to handle. The advantages also include increased versatality ,higher working dilution of primary antibody, secondary antibodies against primary antibody of different species and easy to prepare.

Avidin biotin techniques:

High affinity binding between biotin and avidin is used in this procedure.Biotin is chemically linked to primary antibody and avidin is conjugated chemically to enzyme.The avidin binds to biotinylated antibody thus localizing the peroxidase moiety at the site of antigen.

Disadvantages of this technique is that, the endogenous biotin produces non specific background staining.

Avidin biotin conjugate procedure:

In this technique primary antibody is added followed by biotinylated secondary antibody and next by preformed complexes of avidin and biotin horse raddish peroxidase conjugate.This is a more sensitive method.

Biotin streptavidin system:

Streptavidin is used in place of avidin. Streptavidin complexes are more stable.

Immunogold silver stain technique:

This is used in ultrastructural immunolocalisation.Gold particles are enhanced by the addition of several layers of metallic silver.The fine silver deposits in the background & create confusion when small amounts of antigen are identified.

Polymeric method:

This technique permits binding of large number of enzyme molecules to a secondary antibody via the dextran backbone. Advantages of this technique are

increased sensitivity, minimized non specific background staining and a reduction of total number of assay steps.

Tissue fixation,processing and antigen retrival techniques:

Tissues for IHC undergo fixation,dehydration and paraffin embedding.

Fixation

This is a critical step, as the preservation of morphology is essential for interpretation of IHC.10% buffered formalin is commonly used because of the following advantages.

1. Good morphological preservation
2. Cheap
3. Sterilize tissues
4. Carbohydrate antigens are better preserved.

The disadvantage of masking of antigens during fixation can be overcome by antigen retrivel techniques.

Antigen Retrievel:

This procedure involves unmasking of the antigens.Following techniques can be used.

1. Proteolytic enzyme digestion
2. Microwave antigen retrivel

3. Microwave and trypsin and antigen retrieval technique.
4. Pressure cooker antigen retrieval.

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was done during the period,June 2010-September 2012.It was carried in two groups, namely, apparently healthy female controls and patients with confirmed diagnosis of carcinoma breast.The study was approved by ethical committee of Stanley Medical College.

STUDY POPULATION

CASES

The study sample comprised of 153 breast cancer patients. Cases were chosen from Department of surgey, medical oncology and radiotherapy, Stanley Medical College and Hospital. Clinical history such as size of tumour ,presence of axillary lymphnodes, metastasis, stage and type of breast cancer was also collected.75 patients were screened for receptor status of estrogen through immunohistochemical assay.

CONTROL SUBJECTS

Controls were recruited from outpatient clinic during their visit in gyneacological department. Healthy ,age-matched women ,without a family

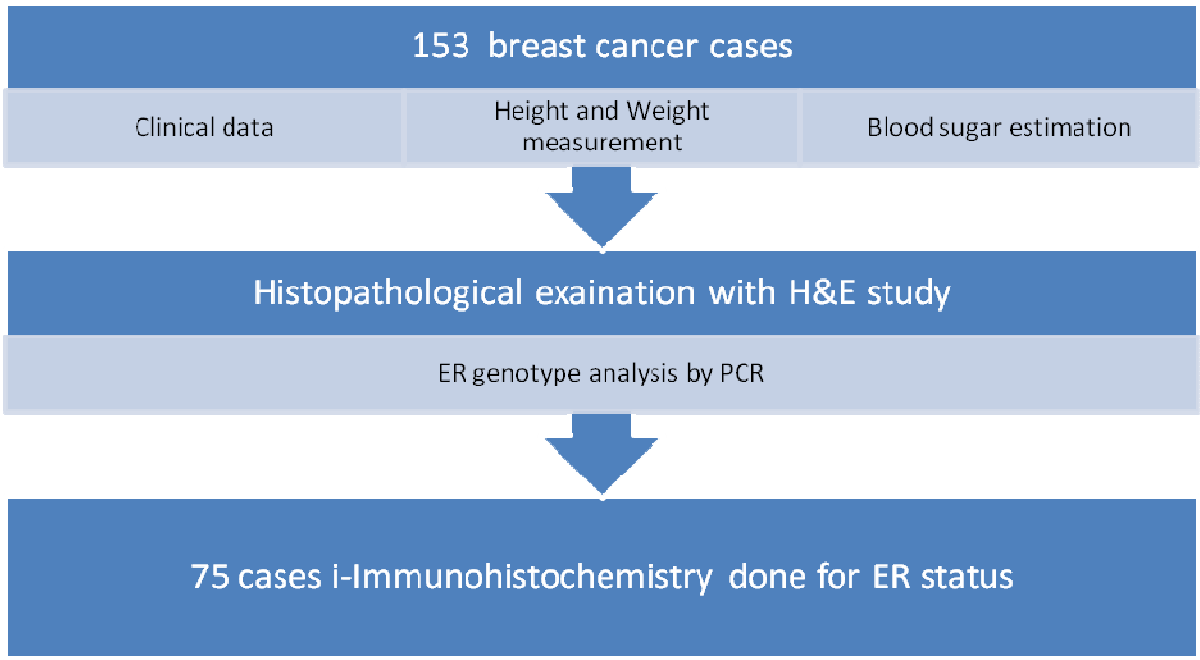
history of breast cancer or any other cancers,were selected to serve as control group.The study sample comprised of 150 controls

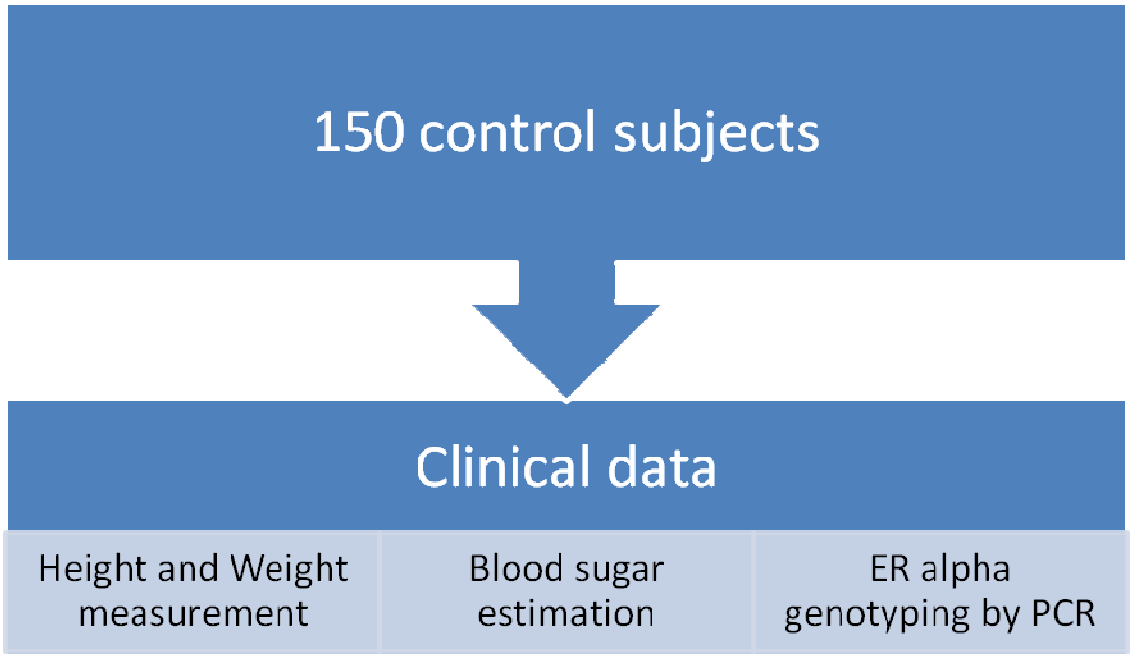
Subjects with amenorrhea ,hormonal disorders,any systemic disease like diabetes mellitus,cardiovascular diseases were excluded from both groups.estrogen receptor alpha gene polymorphism was detected using polymerase chain reaction.

METHOD OF DATA COLLECTION

153 cases were studied and out of these 153 cases 75 cases is selected at random for doing estrogen receptor immunohistochemistry .The tissues so obtained were processed and sections were cut at 5microns. Hematoxylin and eosin staining of the sections were done and studied .

For both the cases and control ER alpha genotype frequency was done by PCR by collecting 5 ml of venous blood. Also random blood sugar was estimated for both cases and control. Height and weight was recorded.


153 breast cancer cases
Clinical data
Height and Weight measurement
Blood sugar estimation
Histopathological exaination with H&E study
ER genotype analysis by PCR
75 cases i-Immunohistochemistry done for ER status


150 control subjects
Clinical data
Height and Weight measurement
Blood sugar estimation
ER alpha genotyping by PCR

METHOD OF TISSUE PREPARATION OF IHC

10% buffered formalin was used for fixing the specimens .The tissues were processed in various grades of alcohol and xylol using automated histokinette.Paraffin blocks were prepared and section of 5 micron thickness were cut in semiautomatic microtome using disposable blades and stained with hematoxylin and eosin.Suitable blocks were chosen for IHC.

Sections for immunohistochemistry were also cut in semiautomatic microtome using disposable blades.Slides coated with chrome alum were used.Sections were subjected to antigen retrival using microwave technique using TRIS EDTA(ph 9.2) buffer solution and then treated by HPR(horse radish peroxidase) polymer technique.

HRP POLYMER TECHNIQUE

The coated slides were taken through the following stages

1. Treatement with peroxidase block –for inhibiting endogenous peroxidases in the tissue for 20 minutes

2. Wash with TRIS buffer for 5 minutes

3. Application of power block –blocks non specific antigen antibody reaction- 20 minutes
4. Blot dry the excess power block
5. Application of primary antibody for 60 minutes
6. Wash in TRIS buffer for 5 minute thrice
7. Application of superenhancer for 30 minutes which enhances the final reaction product by increasing the sensitivity of antigen antibody reaction.
8. Application of SS label –secondary antibody from the goat with the tagged horse radish peroxidase enzyme for 30 minutes
9. Wash thrice in TRIS buffer
10. Application of DAB (Diaminobenzidine) chromogen for 5 minutes –this is cleaved by the enzyme to give the coloured product at the antigen sites
11. Wash in distilled water for 5 minutes
12. The slides were counterstained with hematoxylin
13. Air dried and mounted with DPX(Distrene diputyl pthalide in Xylol)

METHOD OF SCORING FOR ER (36)

Q SCORE

Score for intensity

0-No staining

1-Weak staining

2- Moderate staining

3-strong staining

Score for proportion of positivity

0-No staining

1-<1% of nuclei staining

2-1%-10% of nuclei staining

3-11%-33% of nuclei staining

4-33% -66% of nuclei staining

5-67%-100% of nuclei staining

The scores were summed to give a maximum score of 8.Scores 2 or less are regarded as ER negative.

Sample collection for ER alpha gene polymorphism :

5ml of peripheral venous blood was withdrawn under sterile conditions with disposable syringes from all the cases and controls of this study, and transferred to EDTA tube and mixed thoroughly.EDTA tube was centrifuged at 2000rpm for twenty minutes to get the buffy coat for DNA extraction.

BUFFY COAT PREPARATION

Buffy coat was separated by centrifugation of EDTA tubes at 2000 revolution for 20 minutes.Buffy coat was transferred to 2ml eppendorf and was used for DNA extraction.DNA extraction of some samples were done by chemical method

DNA EXTRACTION BY MODIFIED HIGH SALT METHOD

RBC LYSIS

1.400µL of buffy coat in a 2ml eppendorf was mixed with 1.6ml of 0.17M ammonium chloride and mixed by inversion until red cells are lysed for about 10 minutes

2.The cells were centrifuged at 4000rpm for 10 minutes

3.The white pellet was washed with 800µL of 0.17M ammonium chloride solution .The procedure was repeated till a clear white cell pellet was obtained

WBC LYSIS

To the pellet 500µL of TKM I solution was added .It was centrifuged at 10,000 rpm for 10 minutes

NUCLEAR LYSIS

1.Supernatant was discarded.To the pellet 500µL of TKMII solution was added. 300µL of 6M Nacl and 50µL of 10% SDS was then added.

2.The solution was mixed well & centrifuged at 10000rpm for 10 minutes.

3.Supernatant .was transferred to 1.5ml eppendorf

DNA precipitation

1.To the supernatant double the volume of 100% ethanol was added

2.The sample was stored at -20*c for 1 hour

3.Then it was centrifuged at 10,000rpm for 2 minutes at 4*C in a refrigerated centrifuge.

4.The supernatant was discarded.To this 500µL of 70% ethanol was added .The pellet was mixed and centrifuged at 10,000rpm for 10 minutes at 4*C.

5.Supernatant was discarded and the pellet is air dried

STORAGE

To the pellet 30µL OF LTE buffer is added and the extracted DNA is stored at-20*C for future use.

IDENTIFICATION:

Extracted DNA was identified by 1% agarose gel electrophoresis and comparison with a known molecular weight 1 kb DNA ladder

POLYMERASE CHAIN REACTION:

1300 bp fragment of ESR 1 gene was amplified using Forward primer-TTAGAAAAGCAAAACATGCACTC

Reverse primer-GCCACCCTATCTGTATCTTTTCC(from Helini Biomolecules,Chennai)

Primer Reconstitution:

Primers are supplied in lyophilized form. Autoclaved distilled water was used to prepare 100x concentrations i.e..10 times the molecular weight of primer was the volume of water required to prepare 100x concentrations which is 100µ molar solution.

From this stock solution 10x concentration was prepared as per the working solution for PCR.

MASTER MIX:

Master mix consist of basic components necessary for PCR

- Reaction buffer consisted of TrisHcl-10mM at pH 8.3 KCL-50mM.
- MgCL2-1.5mM acts as catalyst
- dNTP’s were used in a concentration of 2.5mM each.
- Taq polymerase in a concentration of 1.5U

Primers were used in a concentration of 10pmol and DNA was used in a concentration of 200ng.

PCR was carried out in a reaction volume of 25µL with the following components.

PCR master mix-12.5 µL

Forward primer-1.0 µL

Reverse pimer-1.0 µL

DNA-1.0 µL

Distilled water-9.5 µL

Total-25 µL

Amplification was carried out in an Applied Biosystems thermal cycler with the following cycling conditions.

- Initial denaturation-94°C-5 min
- 30 cycles of
    - Denaturation-94°C-30 sec
    - Annealing-55°C-1min
    - Extension-72°C-1 min 30sec
- Final extension at 72°C-5 min

Amplified product –amplicons of 1300bp was identified by 2% agarose gel electrophoresis by comparison with a known 100bp DNA ladder

AGAROSE GEL ELECTROPHORESIS

- PCR product was run on 2.0% agarose gel in a 30 mL agarose cast as follows:0.60g of agarose was weighed and dissolved in 30mL of TAE bufffe with a pH of 8.0
- It was microwaved for 60secs,cooled and 1.5 µL of ethidium bromide(10mg/ml) is added .It is poures into a cast and allowed to solidify for 15 min before it is kept in the electrophoresis tank.
- 10 µL of PCR product is loaded onto wells and 10 µL of 100bp DNA ladder is loaded onto single well as a marker.It is electrophoresed for 30 minand visulaised under UV illumination.

RESTRICTION DIGESTION OF PCR PRODUCTS

ESR1 polymorphism was detected by digestion of the PCR amplified product with Pvull restriction enzyme followed by run in 2% agarose gel electrophoresis.

Principle of Pvull enzyme digestion

- P allele does not have the restriction site hence will yield a 1300bp fragment
- p allele has the restriction site,hence gets cleaved to give 864bp and 436bp fragments.
- Heterozygous individuals (Pp) have 1300bp,864bp,436bp fragments.
- Analysis was done using a 100bp DNA ladder.

Procedure:

- 10 µL of PCR product is aliquoted to an eppendorf and 1U of Pvull enzyme is added.The entire procedure is carried out in ice.The contents are mixed thoroughly.
- The eppendorf is then placed in 37°C waterbath for 2 hours and reaction is stopped by adding 5 µL of gel loading dye and mixed thoroughly.
- Restriction digested product is subjected to 2.5% agarose gel electrophoresis for genotyping.

STATISTICAL ANALYSIS

1. Allele frequencies were calculated by allele counting.
2. Genotype frequency distribution between cases and controls were compared with x^2 test for 2x2 contigency table
3. Association between ER alpha genotype and ER status is compared with Fischer's Extract test.
4. Odd's ratio was calculated to know the correlation of stage of tumour,BMI with genotype distribution.
5. Age and blood sugar levels are compared between control subjects and patients by student t test.

OBSERVATION AND RESULTS

RESULTS

153 breast cancer patients and healthy controls were analysed for genotype distribution of Pvll polymorphism of the ERαgene. The genotype distribution was studied with respect to risk confounding factors such as menopausal status ,stage of the tumour and BMI.75 patients were studied with respect to estrogen receptor status.The results found are as follows:

TABLE 1:Genotype distribution of ER α GENE in breast cancer patients and controls

	PP **n(%)**	**P**p n(%)	pp n(%)
Patients 153	52 (34%)	66 (43.1%)	35 (22.9%)
Control 150	32 (21.3%)	76 (50.7%)	42 (28%)

X^2=6.07

p<0.05

OR for **PP** Vs **P**p-1.87(CI-1.08<OR<3.24)

PP Vs pp-1.95(CI-1.04<OR<3.66)

Pp Vs pp-1.04(CI-0.59<OR<1.82)

The allele frequencies were PP=84,Pp=142,pp=77.this is found to be in hardy Weinberg equilibrium , -1.17,pvalue is 0.28

PP genotype was more frequent among cases(34%) when compared to controls (21.3%) with x^2 value 6.07 and p value<0.28.

TABLE 2: Pvull polymorphism of ER alpha gene and Estrogen receptor status in breast cancer patients:

	Total	**PP**	**P**p	pp
ER POSITIVE	45	20 (44.4%)	23 (51.1%)	2 (4.4%)
ER NEGATIVE	30	6 (20%)	15 (50%)	9 (30%)

Fisher's Exact test=10.78

p<0.01

OR for **PP** Vs **P**p-2.17(CI-0.70<OR<6.66)

PP Vs pp-15.00(CI-2.52<OR<89.23)

Pp Vs pp-6.90(CI-1.30<OR<36.45)

Table shows the frequency of PP genotype was elevted in patients positive for ER(44.4%) as compared to those who were ER negative(23.7%). The OR for genotype PP Vs pp was 15(CI-2.52<OR<89.23).

TABLE 3:Pvull polymorphism of ER α gene and Menopausal status in breast cancer patients

	PP n(%)	**P**p n(%)	pp n(%)
Premenopausal n=79	33 (41.8%)	35 (44.3%)	11 (13.9%)
Control N=74	19 (25.7%)	31 (41.9%)	24 (32.4%)

X^2=8.68

p<0.05

OR for **PP** Vs **P**p-1.54(CI-0.73<OR<3.23)

PP Vs pp-2.46(CI-1.04<OR<5.83)

Pp Vs pp-3.79(CI-1.53<OR<9.41)

Premenopausal women had elevated frequency of PP genotype(41.8%) as compared to postmenopausal women (25.7%) with x^2-8.69,p<0.05. the OR for genotype PP Vs pp was 3.79(95% CI-1.53 to 9.41).The OR for genotype Pp Vs pp was 2.46(95% CI -1.04 to 5.83)

TABLE:4 Pvull polymorphism of ER αGENE and stage of breast cancer patients

	PP n(%)	**P**p n(%)	pp n(%)
Early satge 1&2 n=84	38 (42.2%)	36 (42.9%)	10 (11.9%)
Advanced stagel N=69	14 (20.3%)	30 (43.5%)	25 (36.2%)

X^2=16.74

p<0.01

OR for **PP** Vs **P**p-2.26(CI-1.04<OR<4.94)

Pp Vs pp-3 (CI-1.25<OR<7.23)

PP Vs pp-6.79 (CI-2.61<OR<17.7)

pp genotype was significantly elevated in advanced stage breast cancer(36.2%) when compared to patients with early stage (11.9%).PP genotype frequency was significantly elevated in early stage (42.2%) as compared with advanced stage(20.3%). With X^2=16.74 p<0.01.The OR for genotype Pp Vs pp-6.79 (95%CI-2.61<OR<17.7).The OR for genotype PP Vs pp-3 (95% CI-1.25<OR<7.23).The OR for genotype PP Vs Pp-2.26(95% CI-1.04<OR<4.94)

TABLE:5- Pvull polymorphism of ER αGENE and BMI in breast cancer patients

	PP n(%)	**P**p n(%)	pp n(%)
BMI<30 n=81	31 (38.3%)	34 (41.9%)	16 (19.8%)
BMI>30 N=72	21 (29.2%)	32 (44.4%)	19 (26.4%)

X^2=1.72

p>0.05

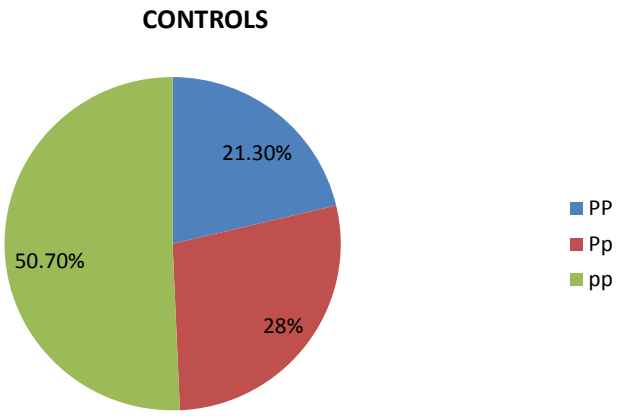
OR for **PP** Vs **P**p-1.39(CI-0.67<OR<2.89)

PP Vs pp-1.75 (CI-0.74<OR<4.17)

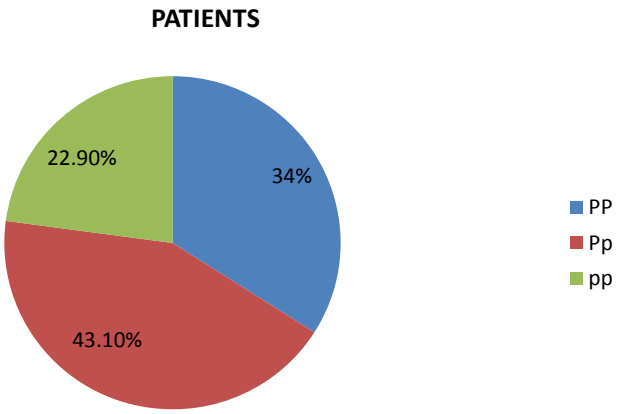
Pp Vs pp-1.26 (CI-0.55<OR<2.87)

It was found that there was no significant difference in genotype distribution among obese and non obese cases (x^2-1.71,p>0.05)

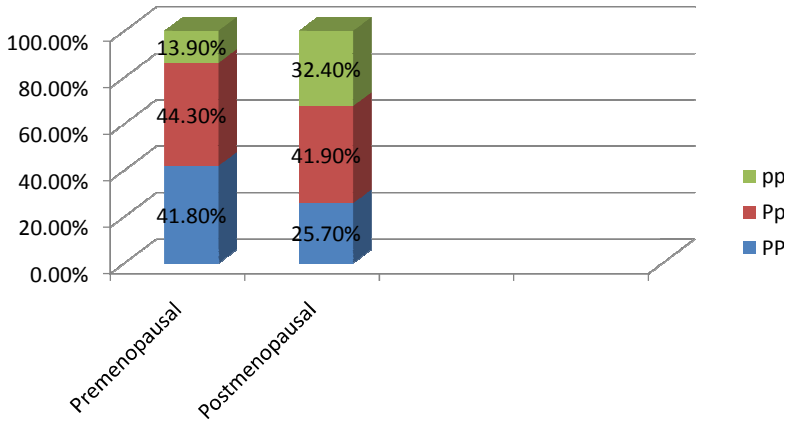
PIE CHART SHOWING GENOTYPE DISTRIBUTION AMONG CONTROLS



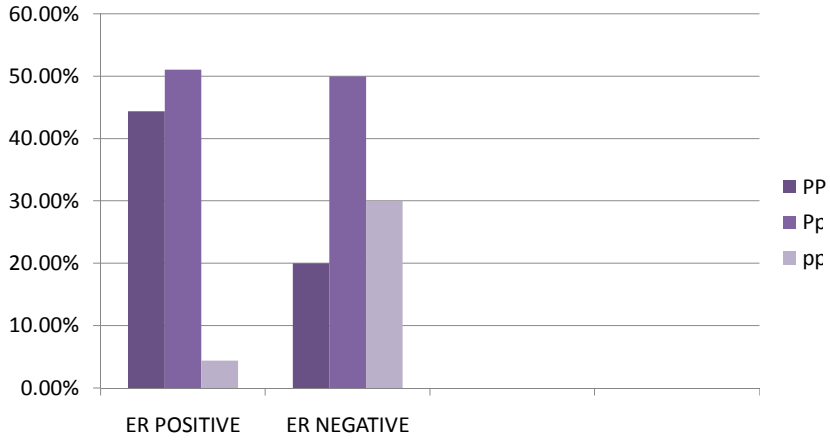
PIE CHART SHOWING GENOTYPE DISTRIBUTION AMONG PATIENTS



Bar diagram showing comparison between genotype distribution among Pre and Postmenopausal women with Breast cancer



Pie diagram showing comparison between genotype distribution and ER status in patients with breast cancer



Bar diagram showing genotype distribution among patients with Early and Advanced stage of Breast cancer

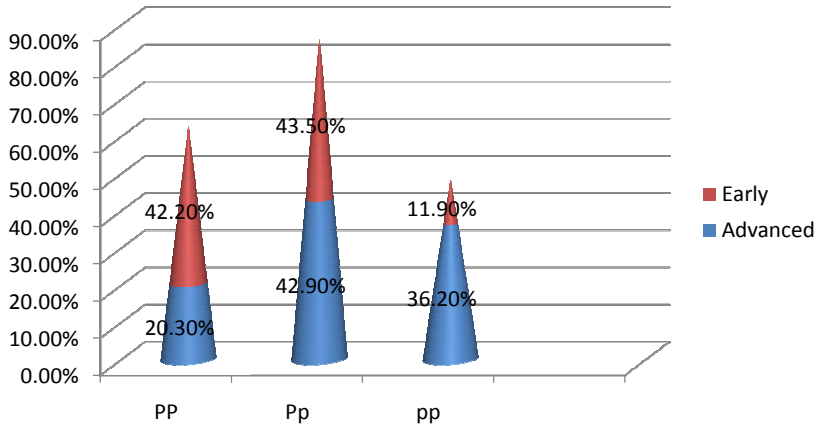


FIGURE : 1 Modified Radical mastectomy C/S -4x3cm grayish white firm mass in the lower outer quadrant

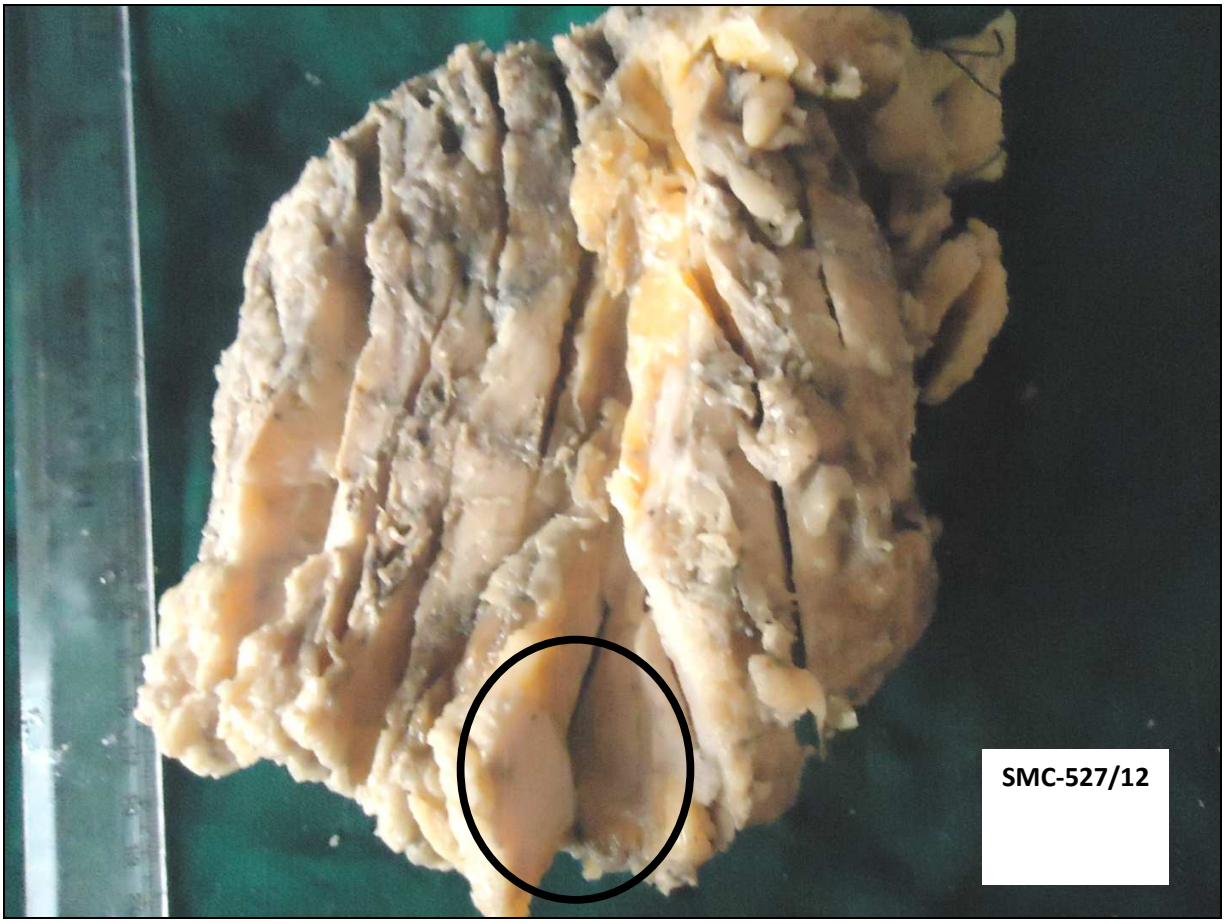


FIGURE : 2 Modified Radical Mastectomy.C/S-3x3cms grey white firm growth in lower quadrant

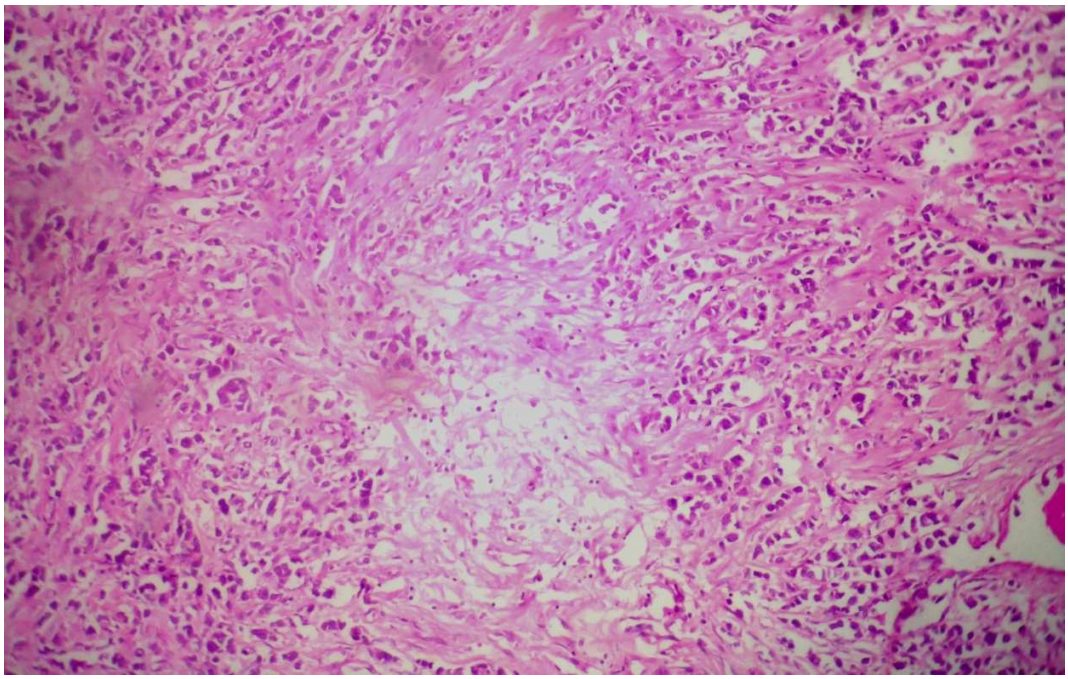


FIGURE: 3 H &E –Infiltrating Ductal Carcinoma NST -10X

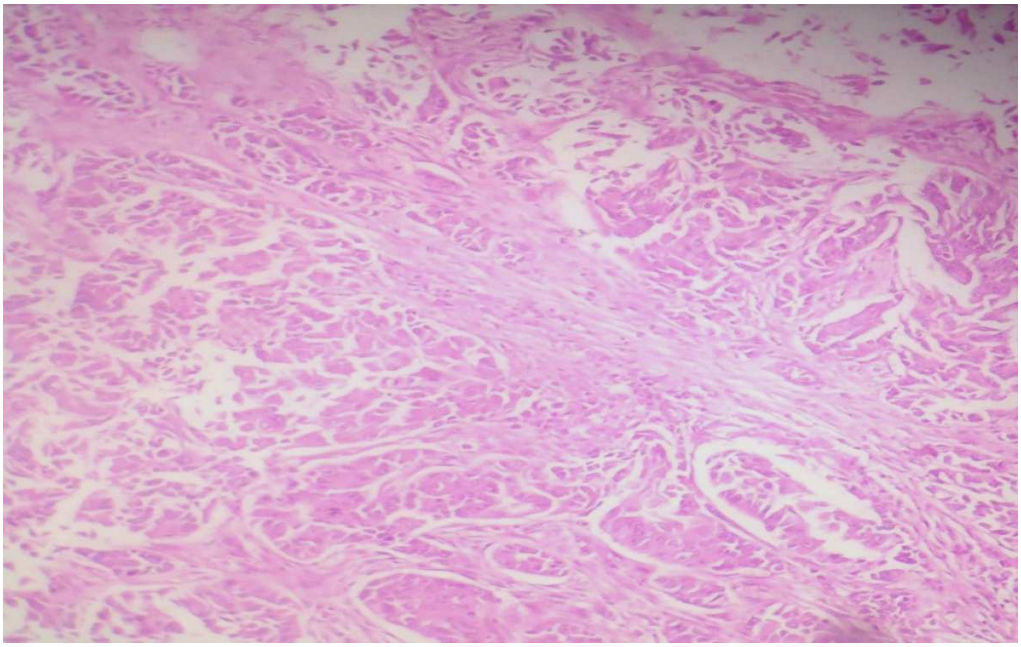


FIGURE: 4 H & E – Infiltrating ductal carcinoma NST-10X

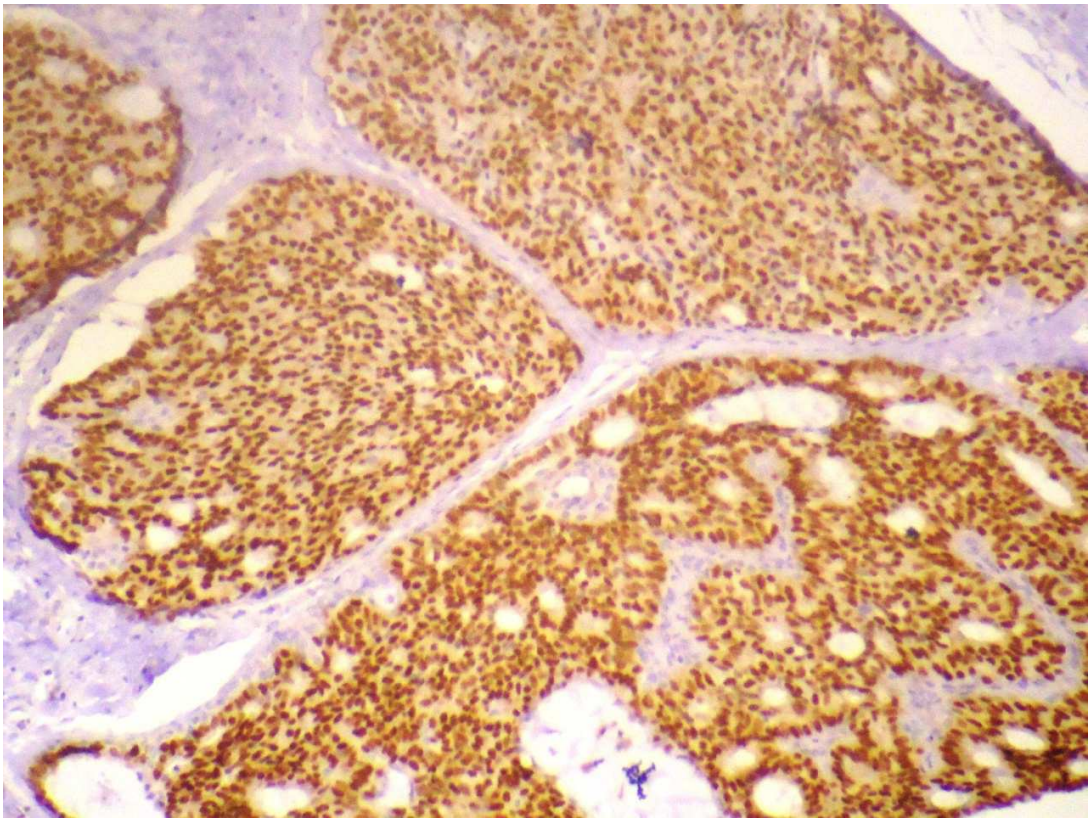


FIGURE: 5 INVASIVE DUCTAL CARCINOMA SHOWING ER POSITIVITY -10X

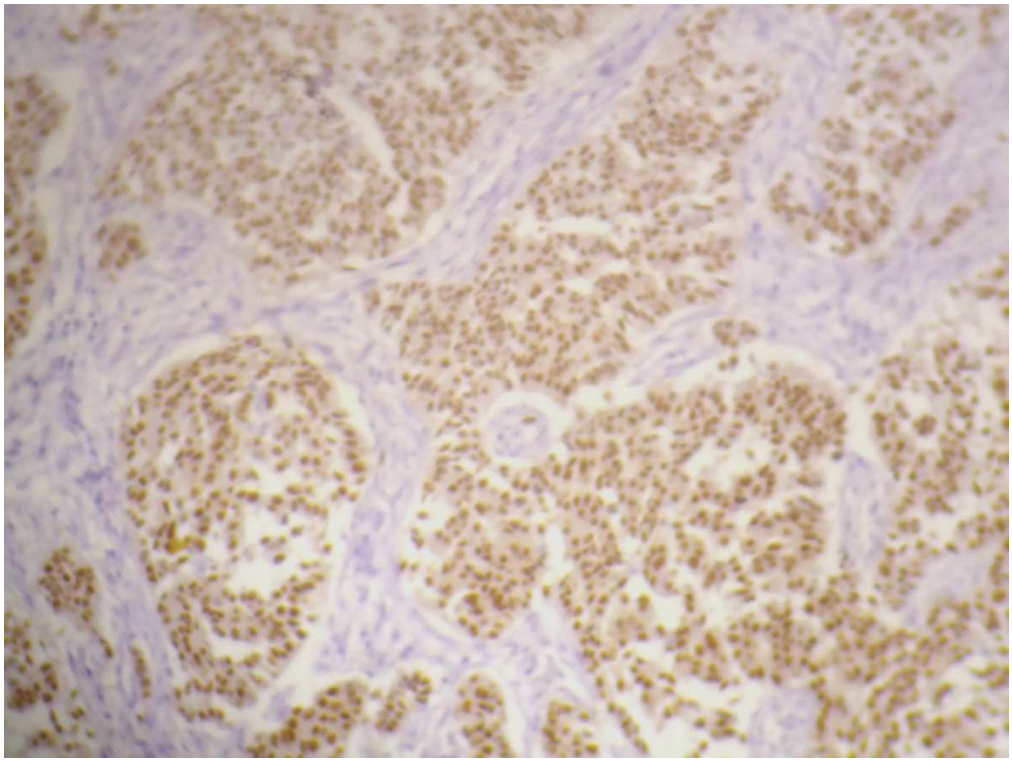


FIGURE: 6 INVASIVE DUCTAL CARCINOMA NST SHOWING ER POSITIVITY-10X

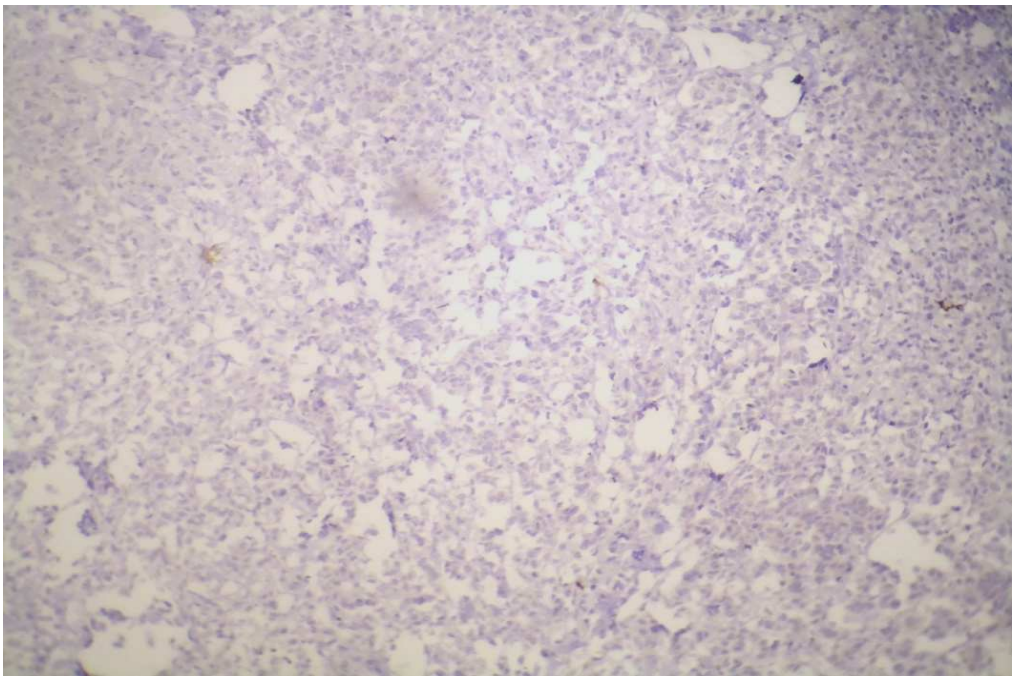


FIGURE: 7 INFILTRATING DUCTAL CARCINOMA NST SHOWING ER NEGATIVITY-10X

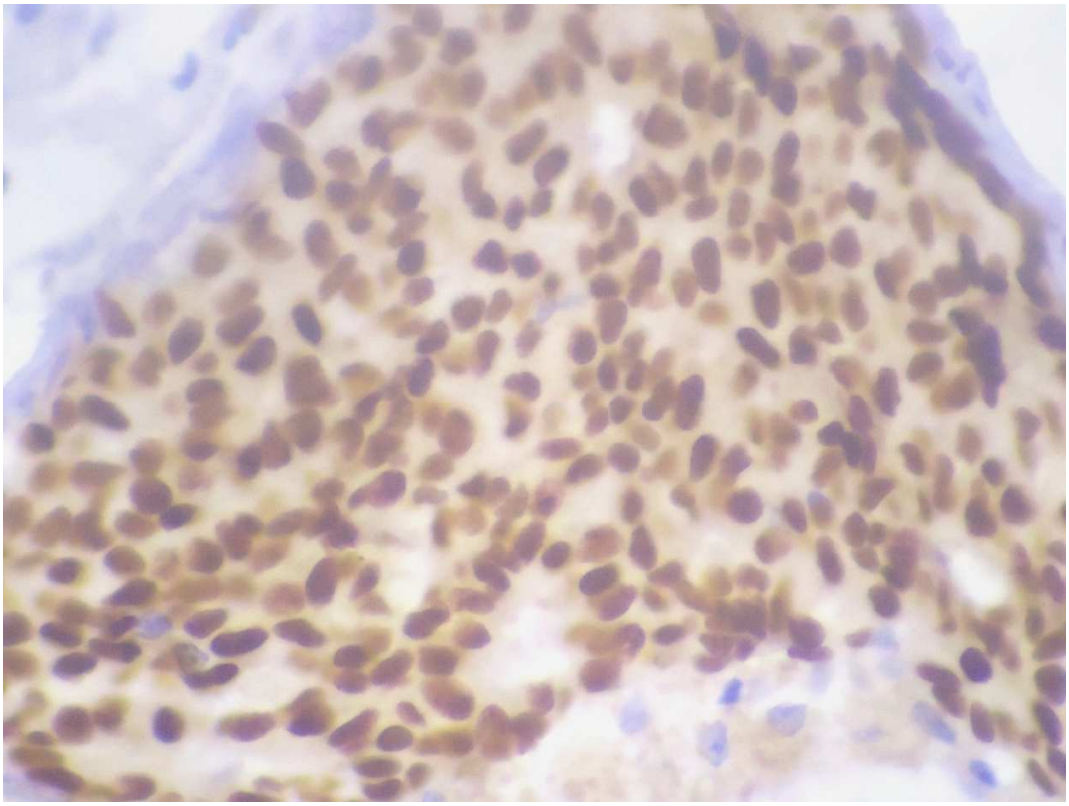


FIGURE: 8 INFILTRATING DUCTAL CARCINOMA NST SHOWING ER POSITIVITY-40X

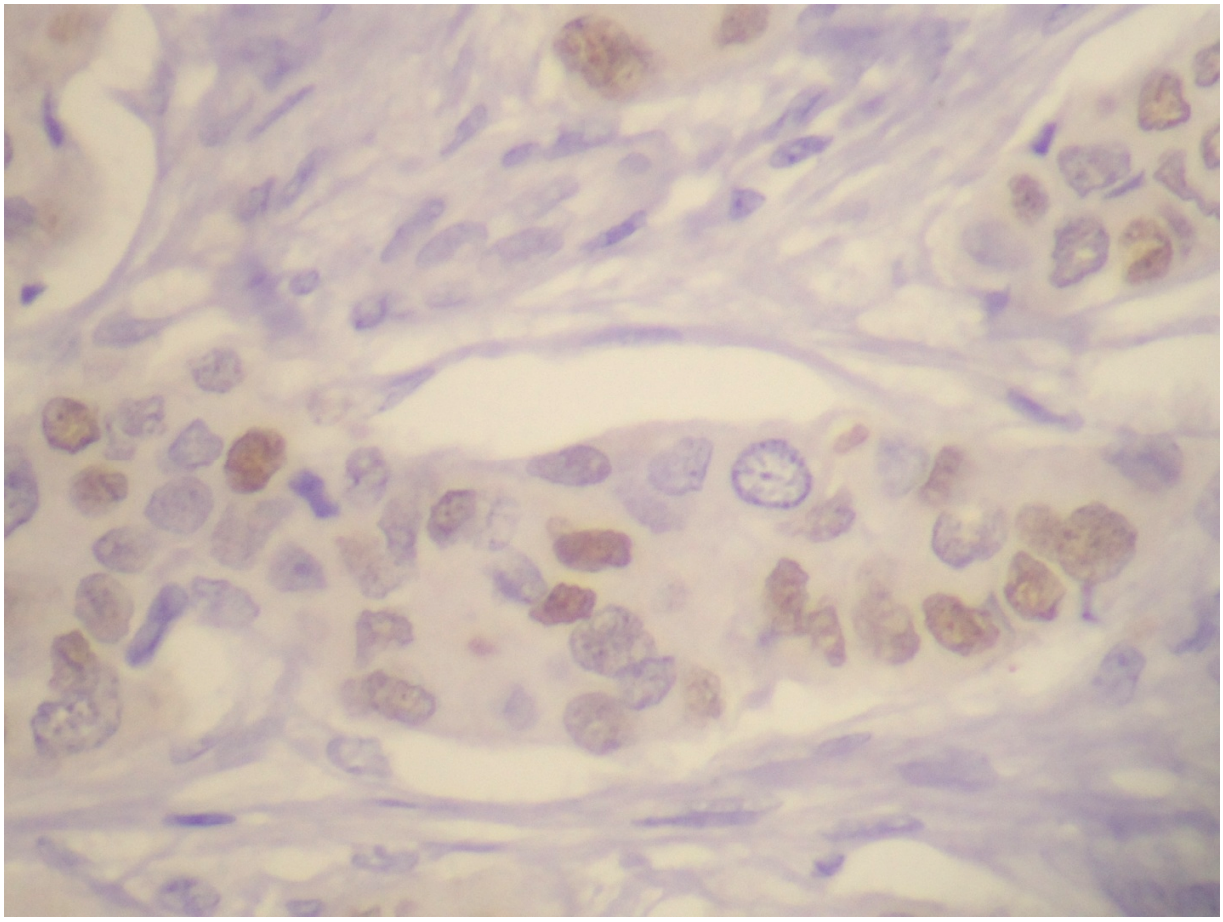


FIGURE: 9 INFILTRATING DUCTAL CARCINOMA NST SHOWING ER NEGATIVITY-40X

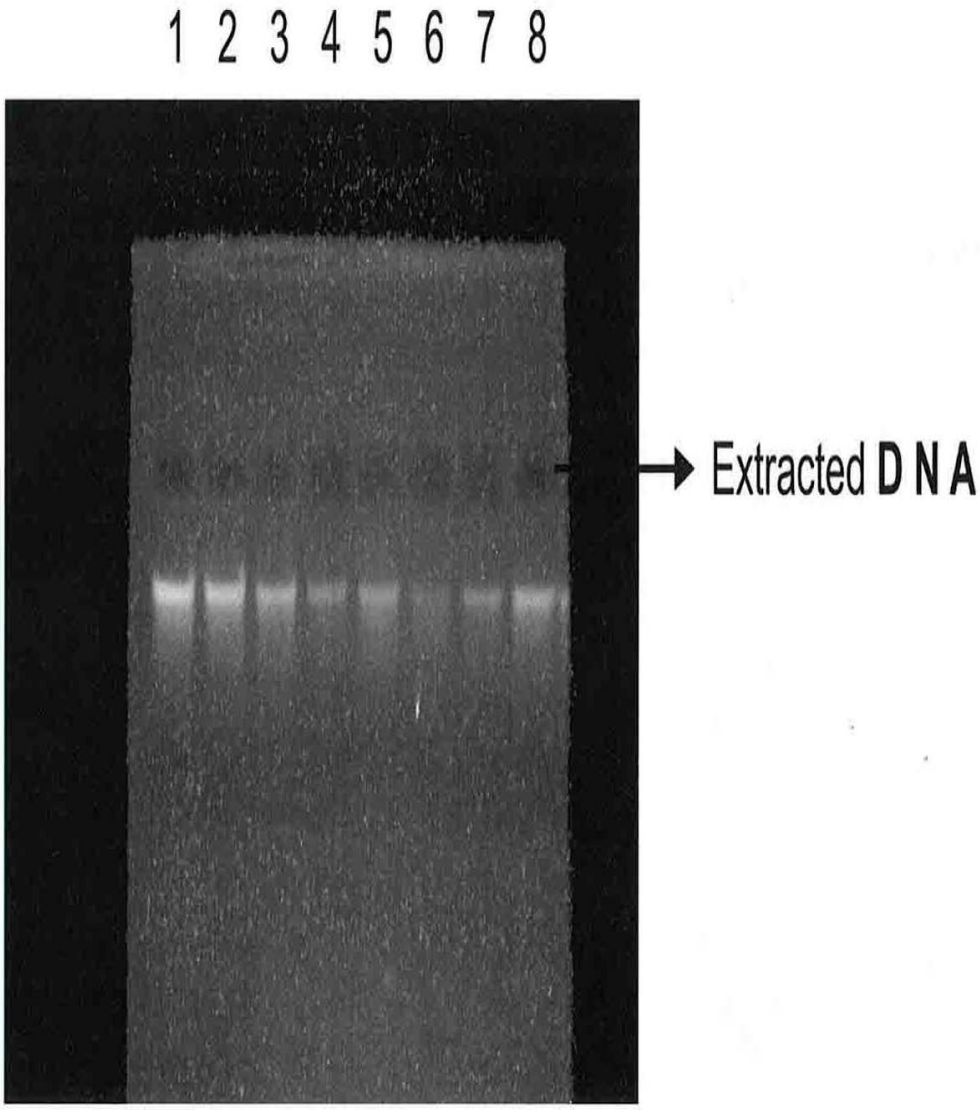


FIGURE: 10 EXTRACTED DNA

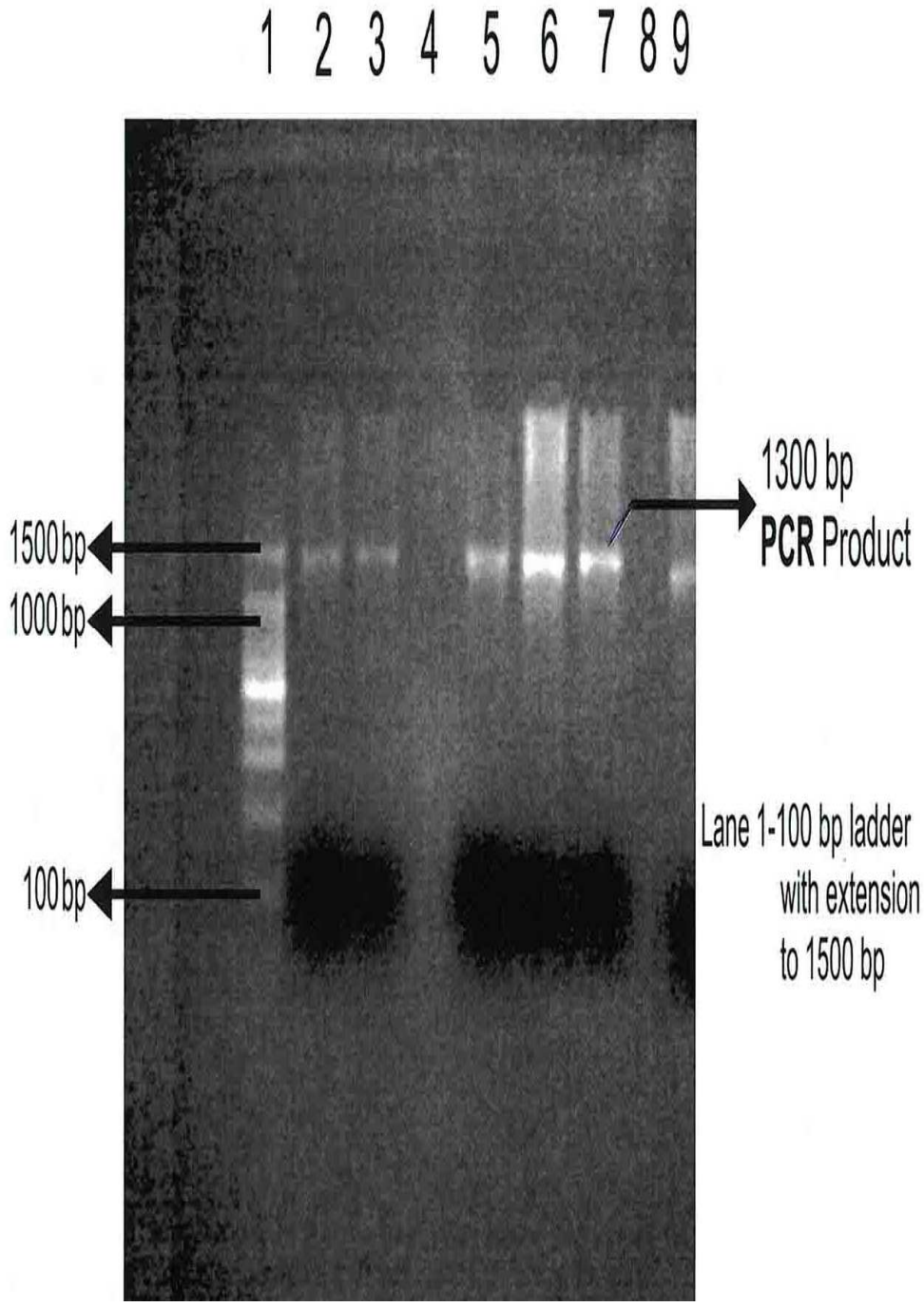
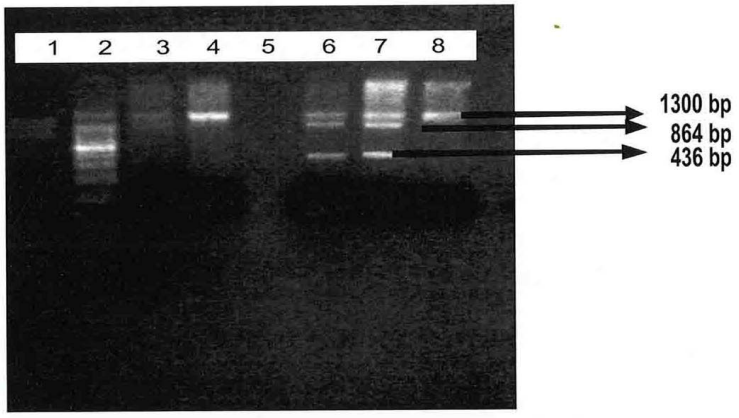
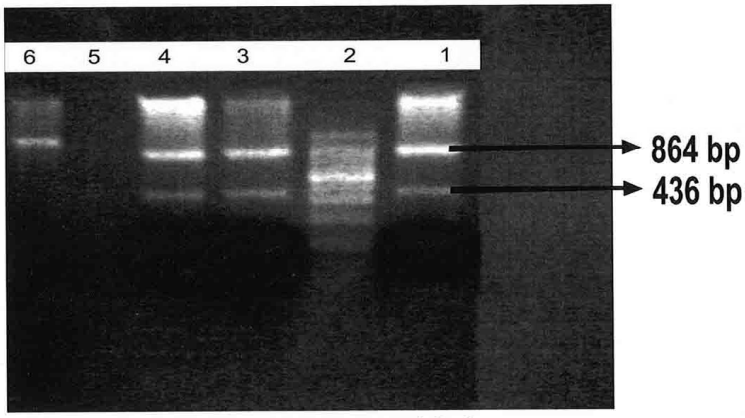


FIGURE: 12 PCR PRODUCT



Lane 2 - 100bp Ladder with Extension to 1500bp
Lane 3,4,8, - PP, Lane 6 ,7- Pp



Lane 2 - Ladder, Lane 1,3,4 - pp
Lane 6 - PP

FIGURE: 11 RESTRICTION DIGESTION FRAGMENTS

DISCUSSION

DISCUSSION

The present study was attempted to evaluate the role of ER alpha Pvull gene polymorphism in the breast cancer development.

The ER alpha gene comprises more than 140kb and has 8 exons and 5 functional domains designated A/B-F. Several sequence variations or single nucleotide polymorphism(SNPs) in ER alpha gene have been identified and found to be associated with either an increased or decreased risk of various diseases(39). The most widely studied polymorphisms of ER alpha were Pvull(T397C) and Xba1(A 351 G) located in intron 1.

Pvull gene polymorphism was studied in various diseases process of various organs. In view of this comprehensive population based study is performed to know the association of ER-α genotype in breast cancer patients.

TABLE-6:Genotype distribution of ER –α gene in breast cancer patient and controls:

Pvull gene polymorphism	Surekha.D et al 2007		Daehee kang et al 2002		Present study	
	Cases n=249	Control n=248	Cases n=201	Control n=195	Cases n=153	Control n=150
PP n(%)	88(35.3)	49(19.8)	35(17.5)	26(13.3)	52(34)	32(21.3)
Pp n(%)	93(37.3)	103(41.5)	91(45.3)	105(53.9)	66(43.1)	76(50.7)
pp n(%)	68(27.3)	68(27.3)	75(37.3)	64(32.8)	35(22.9)	42(28)

Table show the Pvull genotype frequency of the ER-α gene in both breast cancer cases and controls.

According to the study conducted by Surekha.Det al at Nizams instituite of medical sciences,Hydrebad ,India, (40) **PP** genotype show increased asssociation in breast cancer cases(35.3%) as compared to controls(19.8%).

According to the results obtained in the present study,the frequency of PP genotype(34%) show statistically significant association in breast cancer cases as compared to controls(21.3%)

In this respect,the present study was consistent with the cited article.this could be because of similar patient profiles shared by both hospital.

According to the study conducted by Daehee kang et al,2002,Seoul national university college of medicine,Korea(41),**PP** and pp genotype frequency was elevated in breast cancer cases than in controls. It could be explained due to different demographic profile.

Sonia .M.boyapati et al ,2005 (42) study on breast cancer survival indicate that there is no association between ER-α genotypes and breast cancer risk in a population based study in Shangai.

Hence **PP** genotype was elevated in cases, hence that this genotype confers risk of breast cancer in our demographic profile

TABLE 7:.Pvull polymorphism of ER-α gene and Estrogen receptor status in breast cancer patients

	Surekha.d et al		**Present study**	
ER Status	ER positive n=89	ER negative n=97	ER positive n=45	ER negative n=30
PP n(%)	21(23.6)	17(17.5)	20 (44.4%)	6 (20%)
Pp n(%)	38(42.7)	43(44.3)	23(51.1%)	15(50%)
pp n(%)	30(33.7)	37(38.1)	2(4.4%)	9(30%)

In the reference article by Surekha.D et al (40) cited the PP genotype (23.6% Vs21%) was increased in ER positive cases .But the statistical difference in Pp genotype status in both cases and controls are small percentage in both study. Hence this difference of both PP and Pp association may be attributed to the difference in number of cases. PP genotype was the predominant genotype in estrogen receptor positive cases.

In the present study the PP (44.4%)and Pp(51.1%) genotype was increasingly associated with ER positive cases than ER negative cases with PP (20%),Pp(50%)genotype

Sonia .M.boyapati et al ,2005(42) study on breast cancer survival also found there is no variation of genotype according to the ER/PR status.

The present study also revealed the pp genotype was increasingly associated with ER negative cases (30%) than in ER positive cases(4.4%)i.In the surekha.D et al study also revelaed the pp genotype frequency is more in ER negative(38.1%) than ER positive cases(33.7%).

TABLE 8:Pvull polymorphism of ER-α gene and menopausal status in breast cancer patients:

	Surekha.d et al		Present study	
	Pre menopausal n=123	Post Menopausal n=125	Premenopausal n=79	Postmenopausal n=74
PP n(%)	28(22.8)	21(16.8)	33(41.8%)	19(25.7%)
Pp n(%)	48(39)	55(44)	35(44.3%)	31(41.9%)
pp n(%)	47(38.2)	49(39.2)	11(13.9%)	24(32.4%)

In the study conducted by surekha.d et al(40) also PP genotype frequency was elevated in premenopausal women(22.8%) than in post menopausal women(16.8%).Hence we can conclude PP genotype was the risk factor in premenopausal women for breast cancer development.

In the present study,the premenopausal women had elevated frequency of PP genotype(41.8%) as compared to postmenopausal women(25.7%).

Kok HS,Van Asselt KM et al(43) ,in their study had suggested that ER-α gene polymorphism in particular,Pvull ,may affect the age of menopause.

Wee AE et al(44) ,in their study suggested that P allele showed a dose effect relationship with a 0.5 year earlier onset of natural menopause per each copy of the P allele

Pvull polymorphism of ER-α gene and stage of breast cancer

In the present study pp genotype frequencies was significantly elevated in advanced stage breast cancer(36.2%) when compared to patients with early stage (11.9%).

Iin the study conducted by surekha et al(40) also the Pp and pp genotype frequency were found to be increased in patient with large tumour size and advanced stage of disease.Hence the presence of p allele frequency elevated in advanced stage disease may confer that the presence of p allele might confer a risk for an aggressive form of disease.

Pvull polymorphism of ER-α gene and BMI in breast cancer patients:

In the present study there was no significant difference in genotype distribution among the obese and non obese cases.

Surekha.d et al study(40) has concluded that P allele frequency was elevated in overweight and obese patients.

SUMMARY AND CONCLUSION

CONCLUSION

1. From our study ,we conclude that PP genotype may be an independent risk factor for the development of breast cancer.

2. It can also be concluded that pp genotype may be associated with ER negative tumour status and therefore leading to aggressive form of tumour.

3. There was no association seen between ER alpha genotype and and BMI.

SCOPE FOR FURTHER STUDY

- Research aimed identifying the association of familial forms of breast cancer with Pvull allele and whether it may be included as a germline risk factor for familial forms of breastcancer

- Further research may be focused upon the hormone levels and its relationship with genotypic variation in patients with breast cancer,so as to provide efficient preventive measures to genetically susceptible population in future.

ANNEXURES

HO histologic Classification of tumours of breast

Epithelial tumours

Invasive ductal carcinoma, not otherwise specified

Mixed type carcinoma

Pleomorphic carcinoma

Carcinoma with osteoclastic giant cells

Carcinoma with choriocarcinomatous features

Carcinoma with melanotic features

Invasive lobular carcinoma

Tubular carcinoma

Invasive cribriform carcinoma

Medullary carcinoma

Mucinous carcinoma and other tumours with abundant mucin

Mucinous carcinoma

Cystadenocarcinoma and columnar cell mucinous carcinoma

Signet ring cell carcinoma

Neuroendocrine tumours

Solid neuroendocrine

Atypical carcinoid tumour

Small cell / oat cell carcinoma

Large cell neuroendocrine carcinoma

Invasive papillary carcinoma

Invasive micropapillary carcinoma

Apocrine carcinoma

Metaplastic carcinomas

Pure epithelial metaplastic carcinomas

Squamous cell carcinoma

Adenocarcinoma with spindle cell metaplasia

Adenosquamous carcinoma

Mucoepidermoid carcinoma

Mixed epithelial/mesenchymal metaplastic carcinomas

Lipid-rich carcinoma

Secretory carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen-rich clear cell carcinoma

carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Lobular neoplasia

Lobular carcinoma in situ

Intraductal proliferative lesions

- Usual ductal hyperplasia
- Flat epithelial atypia
- Atypical ductal hyperplasia

Ductal carcinoma in situ

Microinvasive carcinoma

Intraductal papillary neoplasms

- Central papilloma
- Peripheral papilloma
- Atypical papilloma

Intraductal papillary carcinoma

Intracystic papillary carcinoma

Benign epithelial proliferations

Adenosis including variants

- Sclerosing adenosis
- Microglandular adenosis
- Adenomyoepithelial adenosis

Myofibroblastoma

Fibromatosis (aggressive)

Radial scar / complex sclerosing lesion

Adenomas

- Tubular adenoma
- Lactating adenoma
- Apocrine adenoma
- Pleomorphic adenoma
- Ductal adenoma

Myoepithelial lesions

Myoepitheliosis

Adenomyoepithelial adenosis

Adenomyoepithelioma

Malignant myoepithelioma

Mesenchymal tumours

Haemangioma

Angiomatosis

Haemangiopericytoma

Pseudoangiomatous stromal hyperplasia

Periductal stromal sarcoma, low grade

Inflammatory myofibroblastic tumour

Lipoma

Angiolipoma

Granular cell tumour

Neurofibroma

Schwannoma

Angiosarcoma

Liposarcoma

Rhabdomyosarcoma

Osteosarcoma

Leiomyoma

Leiomyosarcoma **Fibroepithelial tumours**

Fibroadenoma

Phyllodes tumour

Mammary hamartoma

Tumours of the nipple

Nipple adenoma

Syringomatous adenoma

Pagets disease of the nipple

Malignant lymphoma

Diffuse large B-cell lymphoma

Burkitt lymphoma

Extranodal marginal-zone B-cell lymphoma of MALT type

Follicular lymphoma

Metastatic tumours

Tumours of the male breast

Gynaecomastia

Carcinoma

Invasive

In situ

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INSTITUITIONAL ETHICAL COMMITTEE,

STANLEY MEDICAL COLLEGE,CHENNAI-1

Title of the work :Clinicopathological correlation of Estrogen receptor alpha gene polymorphism and Estrogen receptor in breast cancer-A study of 150 cases

Principal investigator : Dr .N. Hemavathy

Designation :PG in M.D.(Path)

Department :Department of Pathology,
Government Stanley Medical College,
Chennai-1

The request for an approval from the Institutional Ethical Committee(IEC) was considered on the IEC meeting held on 12.10.2011 at the Council hall, Stanley Medical College,Chennai-1 at 2PM.

The members of the Committee, the secretary and the Chairman are pleased to approve the propose work mentioned above , submitted by the principal investigator

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of work for which you applied for ethical clearance.
3. You should inform the IEC immediately,in case of any adverse events or serious adverse reaction.
4. You should abide the rules and regulation of the instituition(s).
5. You should complete the work within the specified period and if any extension of time is required,you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

28/10/11

MEMBER SECRETARY,

IEC,SMC,CHENNAI

TABLE SHOWING GENOTYPE, MEOPAUSAL STATUS , AND BMI IN CASES

BIOPSY NO.	AGE/SEX	MENOPAUSAL STATUS	STAGE	ER-α GENO	HEIGHT	WEIGHT	BMI
2411/08	50/F	POSTMENOPAUSAL	IV	**P**p	161	85	32.79
2976/08	65/F	POSTMENOPAUSAL	III	**P**p	155	53	22.06
3018/08	40/F	PREMENOPAUSAL	II	**PP**	143	70	34.23
3024/08	35/F	PREMENOPAUSAL	I	**PP**	151	78	34.21
3056/08	42/F	PREMENOPAUSAL	II	**P**p	147	54	24.99
3174/08	56/F	POSTMENOPAUSAL	II	**P**p	150	67	29.78
3215/08	57/F	POSTMENOPAUSAL	II	**PP**	155	65	27.06
3251/08	52/F	POSTMENOPAUSAL	II	**P**p	154	76	32.05
4712/08	45/F	PREMENOPAUSAL	II	**PP**	163	54	20.32
5139/08	40/F	PREMENOPAUSAL	III	**PP**	139	65	33.64
5438/08	51/F	POSTMENOPAUSAL	II	pp	148	66	30.13
5890/08	57/F	POSTMENOPAUSAL	III	**P**p	150	69	30.67
634/09	50/F	POSTMENOPAUSAL	II	**P**p	155	74	30.8
1004/09	70/F	POSTMENOPAUSAL	I	**PP**	149	56	25.22
1144/09	50/F	POSTMENOPAUSAL	III	pp	161	50	19.29
1158/09	55/F	POSTMENOPAUSAL	II	**PP**	150	56	24.89
1918/09	40/F	PREMENOPAUSAL	II	**PP**	144	48	23.15
2041/09	46/F	PREMENOPAUSAL	I	**P**p	144	63	30.38
2521/09	47/F	POSTMENOPAUSAL	III	**P**p	156	60	24.65
2813/09	55/F	POSTMENOPAUSAL	II	**PP**	155	65	27.06

2916/09	38/F	PREMENOPAUSAL	II	**P**p	153	55	23.5
3027/09	47/F	PREMENOPAUSAL	II	pp	156	76	31023
3081/09	35/F	PREMENOPAUSAL	II	**PP**	157	80	32.46
3099/09	45/F	PREMENOPAUSAL	III	**P**p	144	48	23.15
3264/09	35/F	PREMENOPAUSAL	II	**PP**	148	66	30.13
3246/09	76/F	POSTMENOPAUSAL	III	**PP**	155	75	31.22
3345/09	45/F	POSTMENOPAUSAL	II	**P**p	139	64	33.12
3471/09	63/F	POSTMENOPAUSAL	III	**P**p	145	57	27.11
3518/09	40/F	PREMENOPAUSAL	II	**P**p	153	73	1.18
3685/09	61/F	POSTMENOPAUSAL	IV	pp	151	78	34.21
3843/09	46/F	PREMENOPAUSAL	I	**PP**	150	56	24.89
3901/09	54/F	POSTMENOPAUSAL	II	**PP**	151	65	28.51
4087/09	36/F	PREMENOPAUSAL	II	**PP**	145	67	31.87
5521/09	65/F	POSTMENOPAUSAL	IV	**P**p	157	78	31.64
36/10	54/F	POSTMENOPAUSAL	IV	pp	148	62	28.31
102/10	40/F	PREMENOPAUSAL	III	**P**p	160	57	22.27
315/10	50/F	POSTMENOPAUSAL	II	pp	162	88	33.53
412/10	60/F	POSTMENOPAUSAL	II	**P**p	151	65	28.51
515/10	40/F	PREMENOPAUSAL	II	**PP**	156	56	23.01
769/10	60/F	POSTMENOPAUSAL	III	**P**p	143	66	32.28
989/10	52/F	POSTMENOPAUSAL	II	**P**p	149	68	30.63
1118/10	45/F	PREMENOPAUSAL	III	pp	151	74	32.45
1361/10	30/F	PREMENOPAUSAL	IV	pp	152	71	30.73

1675/10	48/F	PREMENOPAUSAL	II	**P**p	154	76	32.05
1832/10	61/F	POSTMENOPAUSAL	III	**P**p	160	72	28.12
1918/10	55/F	POSTMENOPAUSAL	IV	**PP**	151	57	25
2211/10	63/F	POSTMENOPAUSAL	III	**P**p	151	62	27.19
2410/10	54/F	POSTMENOPAUSAL	II	**P**p	156	55	22.6
2617/10	45/F	PREMENOPAUSAL	III	**PP**	149	54	24.32
2722/10	42/F	PREMENOPAUSAL	III	pp	152	59	25.54
2745/10	61/F	POSTMENOPAUSAL	II	**P**p	152	74	32.03
2829/10	60/F	POSTMENOPAUSAL	III	pp	144	66	31.83
2981/10	51/F	POSTMENOPAUSAL	II	pp	151	64	28.07
3063/103128	30/F	PREMENOPAUSAL	III	**P**p	164	60	22.31
3168/10	35/F	PREMENOPAUSAL	III	pp	156	77	31.64
3250/10	50/F	POSTMENOPAUSAL	III	**P**p	160	56	21.87
3271/10	32/F	PREMENOPAUSAL	II	**PP**	156	83	30.8
3321/10	43/F	PREMENOPAUSAL	III	**P**p	158	64	25.64
3418/10	63/F	POSTMENOPAUSAL	IV	pp	155	74	30.8
3485/10	38/F	PREMENOPAUSAL	II	**PP**	155	74	32.03
3462/10	41/F	PREMENOPAUSAL	I	**PP**	150	56	24.89
3619/10	58/F	POSTMENOPAUSAL	II	**P**p	155	54	22.48
3742/10	35/F	PREMENOPAUSAL	II	**PP**	152	63	27.27
3810/10	39/F	PREMENOPAUSAL	II	pp	154	59	24.88
3864/10	44/F	PREMENOPAUSAL	II	**P**p	161	85	32.79
3889/10	59/F	POSTMENOPAUSAL	III	pp	152	59	25.54

3910/10	71/F	PREMENOPAUSAL	III	**PP**	155	53	22.06
4010/10	42/F	PREMENOPAUSAL	III	pp	155	57	23.73
4023/10	40/F	PREMENOPAUSAL	II	**P**p	156	74	30.41
4184/10	66/F	POSTMENOPAUSAL	IV	**PP**	156	58	23.83
4218/10	57/F	POSTMENOPAUSAL	II	**P**p	157	60	24.34
4301/10	33/F	PREMENOPAUSAL	II	**P**p	156	56	23.01
4330/10	70/F	POSTMENOPAUSAL	II	**PP**	154	76	32.05
4479/10	42/F	PREMENOPAUSAL	II	**P**p	149	69	31.08
5231/10	44/F	PREMENOPAUSAL	IV	**P**p	155	54	22.48
5536/10	68/F	POSTMENOPAUSAL	II	**PP**	157	50	20.28
108/11	64/F	POSTMENOPAUSAL	IV	pp	150	88	39.11
164/11	43/F	PREMENOPAUSAL	III	pp	146	66	30.96
512/11	40/F	PREMENOPAUSAL	II	**P**p	145	68	32.34
741/11	37/F	PREMENOPAUSAL	I	**P**p	156	68	27.94
841/11	72/F	POSTMENOPAUSAL	II	**PP**	151	76	33.33
1271/11	46/F	POSTMENOPAUSAL	III	pp	144	63	30.38
1286/11	57/F	POSTMENOPAUSAL	IV	**P**p	164	60	22.31
1376/11	55/F	PREMENOPAUSAL	III	**PP**	152	74	32.03
1535/11	40/F	PREMENOPAUSAL	II	**PP**	153	73	31.18
1671/11	60/F	POSTMENOPAUSAL	III	pp	154	59	24.88
1831/11	75/F	POSTMENOPAUSAL	IV	**P**p	157	80	32.46
1911/11	44/F	PREMENOPAUSAL	III	**PP**	155	50	20.81
1928/11	42/F	PREMENOPAUSAL	III	**P**p	163	71	26.72

1929/11	40/F	PREMENOPAUSAL	II	**PP**	155	54	22.48
1956/11	35/F	PREMENOPAUSAL	II	**P**p	156	60	24.65
1975/11	41/F	PREMENOPAUSAL	III	**P**p	148	66	30.13
1989/11	65/F	POSTMENOPAUSAL	IV	**PP**	144	48	23.15
1995/11	50/F	POSTMENOPAUSAL	I	pp	160	57	22.27
2011/11	37/F	PREMENOPAUSAL	II	**PP**	144	48	23015
2152/11	48/F	PREMENOPAUSAL	II	**P**p	152	74	32.03
2231/11	40/F	PREMENOPAUSAL	II	**P**p	156	53	21.78
2511/11	62/F	POSTMENOPAUSAL	III	**P**p	143	56	27.39
2578/11	39/F	PREMENOPAUSAL	II	**P**p	151	78	34.21
2671/11	59/F	POSTMENOPAUSAL	II	**PP**	153	59	25.2
2781/11	37/F	PREMENOPAUSAL	II	**PP**	156	77	31.64
2890/11	42/F	PREMENOPAUSAL	II	**P**p	155	75	31.22
3000/11	64/F	POSTMENOPAUSAL	III	**P**p	145	57	31.87
3015/11	40/F	PREMENOPAUSAL	II	**P**p	143	56	27.39
3781/11	67/F	POSTMENOPAUSAL	III	**PP**	149	68	30.63
3812/11	60/F	POSTMENOPAUSAL	IV	pp	152	56	24.24
3819/11	38/F	PREMENOPAUSAL	II	**PP**	147	54	24.99
3858/11	48/F	PREMENOPAUSAL	III	**PP**	154	73	30.78
3901/11	71/F	POSTMENOPAUSAL	IV	**P**p	151	64	28.07
3925/11	48/F	PREMENOPAUSAL	III	**P**p	154	59	24.88
3973/11	36/F	PREMENOPAUSAL	III	**P**p	156	77	31.64
3981/11	65/F	POSTMENOPAUSAL	IV	pp	143	68	33.25

3984/11	72/F	POSTMENOPAUSAL	III	**PP**	143	56	27.39
3989/11	43/F	PREMENOPAUSAL	III	**P**p	147	75	34.71
4012/11	38/F	PREMENOPAUSAL	II	**PP**	163	73	27.48
4128/11	42/F	PREMENOPAUSAL	II	**P**p	168	78	27.64
4142/11	50/F	POSTMENOPAUSAL	II	**PP**	146	67	31.43
4157/11	54/F	POSTMENOPAUSAL	II	**P**p	149	69	31.08
4210/11	36/F	PREMENOPAUSAL	IV	pp	157	56	22.72
4312/11	66/F	POSTMENOPAUSAL	III	pp	161	85	32.79
4388/11	55/F	POSTMENOPAUSAL	III	**P**p	145	57	27.11
4433/11	35/F	PREMENOPAUSAL	II	**PP**	158	77	30.84
4459/11	68/F	POSTMENOPAUSAL	II	pp	156	77	31.64
4467/11	70/F	POSTMENOPAUSAL	III	**P**p	160	78	30.47
4475/11	28/F	PREMENOPAUSAL	II	**P**p	144	48	23.15
4496/11	37/F	PREMENOPAUSAL	II	**PP**	148	66	30.13
4510/11	65/F	POSTMENOPAUSAL	II	**PP**	155	69	28.72
4621/11	60/F	POSTMENOPAUSAL	II	**P**p	152	56	24.24
4636/11	45/F	PREMENOPAUSAL	II	**P**p	154	73	30.78
4645/11	50/F	POSTMENOPAUSAL	II	pp	148	59	26.94
4751/11	55/F	POSTMENOPAUSAL	II	**PP**	149	64	28.83
4881/11	42/F	PREMENOPAUSAL	III	**P**p	145	57	27.11
5013/11	38/F	PREMENOPAUSAL	II	**PP**	149	64	28.83
5112/11	56/F	POSTMENOPAUSAL	III	pp	150	71	31.56
5132/11	40/F	PREMENOPAUSAL	II	**P**p	157	80	32.46

5158/11	38/F	PREMENOPAUSAL	II	pp	163	60	22.58
5251/11	62/F	POSTMENOPAUSAL	III	pp	149	54	24.32
41/12	34/F	PREMENOPAUSAL	II	**P**p	151	72	31.58
108/12	42/F	PREMENOPAUSAL	II	**PP**	152	69	29.86
325/12	39/F	PREMENOPAUSAL	III	**P**p	159	82	32.44
421/12	54/F	POSTMENOPAUSAL	III	pp	159	82	32.44
471/12	72/F	POSTMENOPAUSAL	III	**PP**	145	68	32.34
527/12	40/F	PREMENOPAUSAL	II	**P**p	158	64	25.64
721/12	44/F	PREMENOPAUSAL	II	**PP**	162	81	33.91
940/12	70/F	POSTMENOPAUSAL	III	pp	145	65	30.92
1015/12	65/F	POSTMENOPAUSAL	II	**PP**	145	57	27.11
1387/12	36/F	PREMENOPAUSAL	III	pp	147	75	34.71
1752/12	42/F	PREMENOPAUSAL	II	**PP**	161	50	19.29
1835/12	56/F	POSTMENOPAUSAL	II	pp	163	71	26.72
1911/12	65/F	POSTMENOPAUSAL	II	**P**p	155	75	31.22
1987/12	40/F	PREMENOPAUSAL	III	**P**p	160	55	21.48
2151/12	60/F	POSTMENOPAUSAL	III	pp	163	73	27.48
2243/12	57/F	POSTMENOPAUSAL	II	**P**p	144	66	31.83

S.NO	NAME	AGE/SEX	GENOTYPE	RBS	HEIGHT	WEIGHT	BMI
1	Kalavathi	32/F	**PP**	99	166	78	28.31
2	Yuvarani	40/F	Pp	129	156	72	29.59
3	Meenakshi	42/F	PP	103	178	74	23.36
4	Ambujam	35/F	Pp	135	150	60	26.67
5	Kanmani	46/F	pp	105	153	62	26.49
6	Shanthi	38/F	**PP**	134	178	58	18.31
7	Yasmin	45/F	**PP**	128	164	69	25.65
8	Praveena	30/F	**PP**	105	151	62	27.19
9	Kaviya	43/F	Pp	100	160	78	30.47
10	Pushpa	52/F	pp	132	148	70	31.96
11	Vijaya	40/F	pp	122	156	65	26.71
12	Jayanthi	39/F	**PP**	99	156	70	28.76
13	Nachiarammal	62/F	Pp	111	170	75	25.95
14	Bhuvana	55/F	Pp	124	163	68	25.59
15	Durga	43/F	Pp	109	165	75	27.55
16	Chandrakal	50/F	pp	100	155	55	22.89
17	Prema	34/F	Pp	120	151	58	25.44
18	Rajathi	46/F	**PP**	115	150	62	27.56
19	Archana	37/F	Pp	119	153	50	21.36
20	Kannamal	65/F	pp	125	163	70	26.35
21	Kanagapriya	70/F	Pp	102	148	60	27.39
22	Tamilselvi	45/F	**PP**	117	160	60	23.44
23	Aarthy	48/F	Pp	107	150	62	27.56
24	Krishnaveni	56/F	Pp	110	163	58	21.83
25	Swapna	40/F	pp	128	158	65	26.04
26	Yalini	33/F	Pp	116	156	80	32.87
27	Thahirabanu	66/F	pp	106	155	62	25.81
28	Suseela	52/F	Pp	130	160	85	33.2
29	Bagyam	37/F	**PP**	122	158	62	24.84
30	Padmadevi	60/F	Pp	132	152	65	28.13
31	Maheswari	55/F	Pp	104	155	62	25.81
32	Aanandhi	40/F	pp	109	152	56	24.24
33	Mumtaj	53/F	Pp	121	161	70	27.01
34	Sulochana	50/F	Pp	110	158	64	25.64
35	Selvi	32/F	**PP**	107	150	52	23.11
36	Maharani	64/F	**PP**	112	160	80	31025
37	Lakshmi	41/F	Pp	103	156	51	20.96
38	Rekha	29/F	**PP**	128	150	52	23.11

39	Tamilmani	28/F	**PP**	100	161	58	22.38
40	Gayathri priya	27/F	**PP**	97	153	50	21.36
41	Priyadharshini	26/F	**PP**	111	158	57	22.83
42	Muralidharani	42/F	Pp	129	151	66	28.95
43	Latha	34/F	pp	118	162	60	22.86
44	Kohila	33/F	**PP**	98	168	72	25.51
45	Geetharani	37/F	pp	107	161	58	22.38
46	Kasthuri	35/F	Pp	120	151	66	28.95
47	Vijaya lakshmi	33/F	Pp	116	154	58	24.46
48	Julie	32/F	Pp	119	167	68	24.38
49	Geetharani	31/F	pp	106	165	65	23.88
50	Thilagavathi	55/F	Pp	102	160	68	26.56
51	Parvatham	48/F	Pp	117	170	60	20.76
52	Rajam	36/F	pp	125	156	51	20.96
53	Rajeshwari	32/F	Pp	130	157	62	25.25
54	Narayani	39/F	pp	110	158	84	33.65
55	Kalimagal	47/F	Pp	126	158	57	22.83
56	Hemavathy	44/F	Pp	131	162	60	22.86
57	Sahiladevi	55/F	Pp	113	159	70	27.69
58	Pushpavathy	62/F	Pp	127	155	50	20.81
59	Devi	43/F	pp	134	154	58	24.46
60	Aadhilakshmi	29/F	**PP**	115	158	62	24.84
61	Manjula	33/F	Pp	102	160	67	26.17
62	Jayalakshmi	39/F	Pp	123	163	54	20.32
63	Bhargavi	56/F	pp	121	160	55	21.48
64	Elizabeth	50/F	Pp	107	152	55	23.81
65	Ambujalakshmi	67/F	pp	125	158	92	36.85
66	Jeyashree	43/F	Pp	104	155	49	20.4
67	Sharmilabanu	40/F	pp	110	158	59	23.63
68	Meenakshi	65/F	pp	112	150	52	23.11
69	Kanagapriya	67/F	Pp	109	156	65	26.71
70	Yogalakshmi	60/F	pp	127	160	65	25.39
71	Sridevi	35/F	**PP**	122	160	75	29.3
72	Golda	42/F	Pp	102	150	68	30.22
73	Sumathy	37/F	pp	100	152	56	24.48
74	Hemavathy	55/F	Pp	120	158	70	28.04
75	Lakshmi	63/F	Pp	124	162	65	24.77
76	Neela	54/F	pp	111	155	50	20.81
77	Sujatha devi	42/F	Pp	100	160	48	18.75

78	Saranya	68/F	pp	103	155	68	28.3
79	Devi	34/F	Pp	110	155	65	27.06
80	Subbulakshmi	45/F	Pp	118	159	68	26.9
81	Neelavathy	60/F	**PP**	121	160	48	18.75
82	Swetha	42/F	pp	123	160	54	21.09
83	Thangam	30/F	Pp	113	158	61	24.44
84	Bagyalakshmi	44/F	Pp	109	155	68	28.3
85	Kanagavalli	46/F	pp	112	153	50	21.36
86	Ramani	42/F	**PP**	118	165	60	22.04
87	Rajalakshmi	40/F	Pp	110	160	54	21.09
88	Anuradha	54/F	pp	107	163	58	21.83
89	Vasantha kumari	47/F	Pp	117	160	58	22.66
90	Alamelu	45/F	Pp	112	160	52	20.31
91	Dorathy	50/F	pp	101	156	54	22.19
92	Vasuki	33/F	**PP**	100	155	58	24.14
93	Ramadevi	42/F	**PP**	102	164	65	24.17
94	Abidha suhara	52/F	Pp	120	162	53	20.2
95	Manohari	46/F	Pp	142	158	75	30.04
96	Juliet	45/F	pp	137	160	54	21.09
97	Leela	34/F	Pp	115	154	50	21.08
98	Lalitha	34/F	Pp	140	151	56	24.56
99	Mahalakshmi	29/F	pp	108	160	54	21.09
100	Devikala	35/F	pp	128	154	50	21.08
101	Ambiga	46/F	Pp	130	150	54	24
102	Bagyam	48/F	pp	135	162	74	28.2
103	Seethalakshmi	53/F	Pp	107	155	82	34.13
104	Priyadharshini	39/F	Pp	99	159	65	25.71
105	Sugandhi	62/F	Pp	145	163	61	22.96
106	Veeralakshmi	58/F	pp	138	160	54	21.09
107	Kalpana	46/F	**PP**	128	157	59	23.94
108	Meenakumari	38/F	Pp	131	158	65	26.04
109	Subhashini	40/F	Pp	123	158	62	24.84
110	Agatha	52/F	pp	104	154	60	25.3
111	Shanthi	35/F	**PP**	112	160	53	20.51
112	Vasumathi	37/F	Pp	130	154	62	26.14
113	Dheepa	35/F	**PP**	105	159	65	25.71
114	Kalaivani	44/F	Pp	120	153	75	32.04
115	Geetharani	35/F	pp	109	144	60	28.94
116	Gowri	45/F	**PP**	139	170	69	23.88

117	Chitrapavai	47/F	**PP**	128	159	66	26.11
118	Amudha	40/F	pp	110	160	52	20.31
119	Dhanalakshmi	32/F	Pp	126	164	50	18.59
120	Gandhimathi	35/F	Pp	119	163	58	21.83
121	Mythili	48/F	pp	130	155	75	31.22
122	Menaka	40/F	**PP**	125	158	60	24.03
123	Sangeetha	45/F	Pp	100	164	50	18.59
124	Pavithra	55/F	Pp	103	155	75	31.22
125	Shyamiladevi	42/F	Pp	107	158	54	21.63
126	Vanitha	30/F	**PP**	99	155	60	24.97
127	Yamunadevi	62/F	pp	134	151	56	24.56
128	Shiny	44/F	Pp	117	158	59	23.63
129	Hemavathy	38/F	Pp	123	156	55	22.6
130	Prabhavathy	45/F	pp	134	157	75	30.43
131	Jothilakshmi	35/F	**PP**	121	162	58	22.1
132	Suryalakshmi	65/F	pp	103	158	72	28.84
133	Kanchan	49/F	Pp	128	156	56	23.01
134	Swathi	57/F	Pp	122	155	83	33.3
135	Valarmathi	46/F	pp	115	166	63	22.86
136	Navamani	32/F	**PP**	123	146	45	21.11
137	Andal	45/F	Pp	113	156	68	27.94
138	Sankari	59/F	pp	121	158	76	30.44
139	Sivamalar	29/F	**PP**	111	151	51	22.37
140	Tamilselvi	41/F	Pp	107	159	84	33.23
141	Umamaheshwari	40/F	Pp	102	153	55	23.5
142	Kayalvizhi	35/F	pp	114	155	72	32.47
143	Karpagam	38/F	Pp	99	152	60	25.97
144	Manimozhi	48/F	Pp	101	160	52	20.31
145	Pushpa	55/F	**PP**	103	155	52	21.64
146	Amudha	59/F	Pp	120	162	55	20.96
147	Yogeshwari	44/F	pp	128	155	70	29.14
148	Komalavalli	42/F	**PP**	104	156	53	21.78
149	Meera	40/F	Pp	117	156	56	23.01
150	Padmini	45/F	Pp	115	155	68	28.3

TABLE SHOWING ER ALPHA GENOTYPE AND ER RECEPTOR IN CASES

S.NO.	BIOPSY NO.	AGE/SEX	ER STATUS	ER-α GENOTYPE
1	2411/08	50/F	POSITIVE	**P**p
2	2976/08	65/F	POSITIVE	**P**p
3	3018/08	40/F	POSITIVE	**PP**
4	3024/08	35/F	POSITIVE	**PP**
5	3056/08	42/F	NEGATIVE	**P**p
6	3174/08	56/F	POSITIVE	**P**p
7	3215/08	57/F	NEGATIVE	**PP**
8	3251/08	52/F	NEGATIVE	**P**p
9	4712/08	45/F	POSITIVE	**PP**
10	5438/08	51/F	POSITIVE	pp
11	5890/08	57/F	POSITIVE	**P**p
12	634/09	50/F	POSITIVE	**P**p
13	1144/09	50/F	NEGATIVE	pp
14	1158/09	55/F	POSITIVE	**PP**
15	1918/09	40/F	POSITIVE	**PP**
16	2041/09	46/F	POSITIVE	**P**p
17	2521/09	47/F	NEGATIVE	**P**p
18	2916/09	38/F	POSITIVE	**P**p
19	3081/09	35/F	POSITIVE	**PP**
20	3246/09	76/F	NEGATIVE	**PP**
21	3471/09	63/F	POSITIVE	**P**p
22	3518/09	40/F	POSITIVE	**P**p
23	3685/09	61/F	NEGATIVE	pp
24	3901/09	54/F	POSITIVE	**PP**
25	4087/09	36/F	NEGATIVE	**PP**
26	5521/09	65/F	NEGATIVE	**P**p
27	515/10	40/F	POSITIVE	**PP**
28	769/10	60/F	NEGATIVE	**P**p
29	989/10	52/F	POSITIVE	**P**p
30	1118/10	45/F	NEGATIVE	pp
31	1361/10	30/F	NEGATIVE	pp
32	2211/10	63/F	NEGATIVE	**P**p
33	2410/10	54/F	POSITIVE	**P**p
34	2745/10	61/F	POSITIVE	**P**p
35	3063/10	30/F	POSITIVE	**P**p
36	3168/10	35/F	NEGATIVE	pp
37	3250/10	50/F	NEGATIVE	**P**p
38	3321/10	43/F	POSITIVE	**P**p
39	3864/10	44/F	POSITIVE	**P**p
40	3910/10	71/F	POSITIVE	**PP**
41	4010/10	42/F	NEGATIVE	pp
42	4023/10	40/F	NEGATIVE	**P**p
43	4184/10	66/F	NEGATIVE	**PP**
44	4330/10	70/F	POSITIVE	**PP**
45	4479/10	42/F	NEGATIVE	**P**p

46	5231/10	44/F	NEGATIVE	**P**p
47	4330/10	70/F	POSITIVE	**PP**
48	4479/10	42/F	NEGATIVE	**P**p
49	5231/10	44/F	NEGATIVE	**P**p
50	512/11	40/F	POSITIVE	**P**p
51	741/11	37/F	POSITIVE	**P**p
52	1286/11	57/F	NEGATIVE	**P**p
53	1376/11	55/F	NEGATIVE	**PP**
54	1831/11	75/F	POSITIVE	**P**p
55	1928/11	42/F	NEGATIVE	**P**p
56	1929/11	40/F	POSITIVE	**PP**
57	1956/11	35/F	POSITIVE	**P**p
58	1975/11	41/F	NEGATIVE	**P**p
59	1995/11	50/F	NEGATIVE	pp
60	2011/11	37/F	POSITIVE	**PP**
61	2152/11	48/F	NEGATIVE	**P**p
62	2231/11	40/F	POSITIVE	**P**p
63	2671/11	59/F	POSITIVE	**PP**
64	2781/11	37/F	POSITIVE	**PP**
65	2890/11	42/F	POSITIVE	**P**p
66	3819/11	38/F	POSITIVE	**PP**
67	3925/11	48/F	POSITIVE	**P**p
68	3973/11	36/F	POSITIVE	**P**p
69	4012/11	38/F	POSITIVE	**PP**
70	4142/11	50/F	POSITIVE	**PP**
71	4210/11	36/F	NEGATIVE	pp
72	325/12	39/F	NEGATIVE	**P**p
73	1835/12	56/F	NEGATIVE	pp
74	1987/12	40/F	NEGATIVE	**P**p
75	2151/12	60/F	NEGATIVE	pp